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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

Volume 46, Number 11 November 1958

CONTENTS

	PAGE	RUSS. PAGE
PHYSIOLOGY		
The Role of the Cerebellum in the Performance of Motor Activity. I. M. Gil'man	1285	3
The Effect of Sham Feeding on the Basal Metabolism in Dogs. I. A. Voronenkova	1290	7
The Possibility of Development of Positive and Negative Conditioned Reflexes from Symmetric Areas of the Skin. A. P. Savinskaia	1294	11
The Combined Activity of the Spleen and Other Organs. Communication I. The Effect of Experimental Splenopathy on the Functional State of the Thyroid Gland and the Ovaries. L. I. Geller	1300	17
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
Concerning the Reflex Mechanism of Antibody Formation. A. D. Ado and L. M. Ishimova	1305	22
The Effect of Adrenocorticotropic Hormone on the Development of Experimental Atherosclerosis. A. B. Vinogradskii	1311	28
The Effect of Physical Strain on the Development of Experimentally Induced Atherosclerosis. N. N. Kipshidze	1316	32
Changes in the Visco-Elastic Properties of Muscles in Contractures After Immobilization. G. A. Chetverikova	1322	38
The Mechanism of the Disorders of Respiration in Toxic Forms of Diphtheria. Communication I. The Effect of Diphtheria Toxin on the Functional State of the Respiratory Center in Guinea Pigs. I. P. Garanina	1327	42
Adrenalin-Like Substances in Myasthenia. L. B. Perel'man and E. Sh. Matlina	1331	46
BIOCHEMISTRY AND BIOPHYSICS		
Conversion of β-Alanine in Vivo and in Isolated Liver Tissue. S. E. Severin and U. Wei-Min	1335	51
Lipid Metabolism in the Liver and the Effect of Glutathione on This Process During Excessive Intake of Cholesterin in the Diet. I. B. Fridliand	1339	55
Mitogenetic Analysis of the Protein Structure of Protoplasm. Communication I. Experiments on a Gelatin Model. N. S. Slavina	1342	57
MICROBIOLOGY AND IMMUNITY		
The Competition of Allergic Processes. M. A. Frolova, G. A. Klisenko and L. I. Krasnoproshina	1346	62

CONTENTS (continued)

	PAGE	RUSS. PAGE
The Plasmocytic Reaction and Immunological Laws. Communication II. Immunological Inhibition During Immunization of Rabbits with Tetanus Toxoid. G. V. Shumakova and G. A. Gurvich	1350	66
The Specificity of the Humoral Factors in Transplantational Immunity. E. A. Zotikov	1357	72
The Use of the Method of Anaphylaxis with Desensitization for the Study of the Antigenic Properties of Tumors During Heterotransplantation. V. K. Kozlov	1362	78
Determination of the Serological Activity of the Desoxyribonucleoproteins (DNP) of Human Gastric Carcinoma Tissues after Adsorption on Cotton Fabric. N. A. Shagunova and D. G. Grigor'ian	1368	84
Antigenic Differentiation of the Grey and White Matter of the Brain in Man. N. I. Kuznetsova and P. N. Kosiakov	1371	87
ONCOLOGY		
Distinctive Features of the Development of M-1 Tumors in Rats During Changes in the Functional Condition of the Nervous System. A. N. Kursheva	1375	91
Changes in the Properties of the Serum During Growth of a Brown-Pearce Carcinoma in Rabbits. M. N. Nilovskii	1379	94
The Effect of High Intensity Ultrasound on the Growth and Metastasization of Inoculated Brown-Pearce Tumors in Rabbits. N. P. Dmitrieva	1383	97
The Effect of Aseptic Proliferative Inflammation on the Localization and Growth of Spontaneous Tumors of the Mammary Glands in Mice of the High Cancer C_3HA Strain. M. D. Podil'chak	1389	103
EXPERIMENTAL BIOLOGY		
Factors of Osteogenesis and the Secretory Function of Transitional Epithelium. A. Ia. Fridenshtein	1394	108
The Mitotic Behavior of the Epithelium of the Uterus at Different Stages of the Sexual Cycle in Mice. O. I. Epifanova	1399	113
METHODS		
A Method of Oncometry of the Brain. K. N. Badmaev	1403	118
A Method of Measurement of Muscular Tone in Animals. I. A. Slavutskaia	1408	121
A Method of Continuous Recording of the Blood Flow in the Brain in Experiments of Short and Long Duration. D. I. Parolla	1411	123
A Modification of the Method of Differential Manometry for Registration of the Volume Velocity of the Blood Flow. A. V. Dokunin	1414	126

PHYSIOLOGY

THE ROLE OF THE CEREBELLUM IN THE PERFORMANCE OF MOTOR ACTIVITY

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(Received April 20, 1957. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The question of the mechanism involved in the effect of the cerebellum on the course of coordinated movements when the cerebral cortex is intact has not so far been fully clarified, although there are several interesting references to this problem [1, 4-6, 9-11, 13-21].

Our investigations were devoted to the study of the role of the cerebellum in the coordination of movements which arise under the influence of cerebral cortical stimulation.

EXPERIMENTAL METHOD

Experiments were performed on 80 cats. The motor area of the left cerebral hemisphere and the paramedian lobe of the right cerebellar hemisphere were exposed under ether anesthesia. It is known that the latter structure is particularly closely associated with the hind limb areas of the contralateral cerebral hemisphere. The distal ends of two antagonist muscles of the right hind limb – semitendinosus and quadriceps femoris (rectus) – were dissected and connected by a thread to the angular levers of a double myograph. The experiments, conducted under urethane anesthesia, consisted of investigation of the effect of changes in the cerebellum on the motor effect of induction-current stimulation of appropriate points on the cerebral cortex.

The cerebellum was stimulated for 2-7 seconds by direct current (anode or cathode) of subthreshold strength, the diameter of the stimulating electrode being 0.3 mm. The animal was allowed to rest for not less than 7-10 minutes after each stimulation of the cerebellum. Usually the character of the coordination relations between antagonist muscles was determined prior to cerebellar stimulation, during such stimulation, and following it. In a number of experiments cerebellar stimulation was carried out briefly against the background of cerebral stimulation lasting 6-8 seconds: this gave us the opportunity to observe the muscular reaction to cerebral stimulation during the first few seconds without cerebellar stimulation, during the subsequent 2-3 seconds against the background of such stimulation, and finally during the last 2-3 seconds after its termination.

In experiments without general anesthesia, stimulation of the appropriate cortical point usually leads readily to reciprocal reactions in antagonist muscles. Under anesthesia no such constancy of reaction could be observed and simultaneous contraction of both muscles was noted frequently.

EXPERIMENTAL RESULTS

The experiments showed that stimulation of the cerebellum with direct current exerts an influence on the effect of cortical stimulation, and two main types of changes were found to be consistently reproducible in many experiments. The first and more frequently encountered type of change consisted only of enhancement or diminution of the intensity of those muscular reactions which were observed during stimulation of the cerebral cortex (Fig. 1). If, for example, cortical stimulation evoked reciprocal reactions of antagonist muscles, then cerebellar stimulation either increased the reciprocity by increasing the height of contraction of one muscle and the simultaneous enhancement of relaxation in its antagonist, or, conversely, diminished the reciprocity. If, however, cortical stimulation led to the contraction of both muscles, i.e. a unidirectional reaction, then

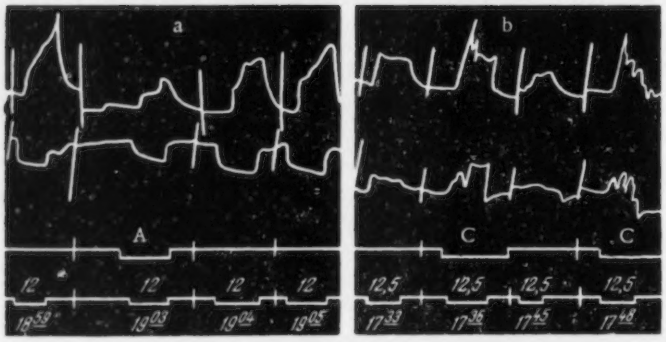

Fig. 1. Change in the intensity of reactions.
a) Diminution of reciprocity (experiment December 4, 1952); b) increase in height of contraction of both muscles (experiment May 13, 1953). Records (from above down): reaction of the quadriceps-muscle reaction of the semitendinosus muscle; marker denoting start and termination of anodic (A) and cathodic (C) direct current stimulation of the cerebellum; marker denoting cerebral cortical stimulation (figures designate the distance between induction coils in centimeters; figures in the last line indicate time).

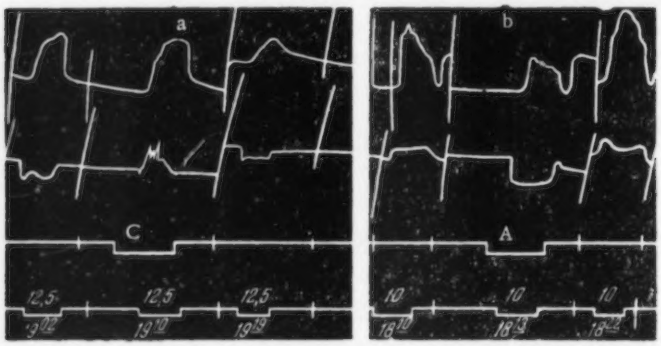

Fig. 2. Change in coordination relations to reverse ones.
a) Transition from reciprocal reaction to contraction of both muscles;
b) appearance of reciprocal reaction instead of contraction of both muscles. Records the same as in Fig. 1.

cerebellar stimulation led to simultaneous change in the intensity of their contraction, which was either enhanced or diminished.

The second type of change manifested itself in a fundamental rearrangement of coordination relations and establishment of new muscle interrelationships – the reverse of those which had been obtained as the result of cerebral cortical stimulation without cerebellar stimulation (Fig. 2). Thus, in cases of initial reciprocal reaction, simultaneous contraction of both muscles took place, since one of them, instead of relaxing, began to show a definite contraction reaction. In cases of contraction of both muscles, cerebellar stimulation was followed by relaxation of one of them while the other continued to contract, i.e. reciprocal relationships came to the fore. In those experiments in which cerebellar stimulation was briefly superimposed on prolonged cortical stimulation, the change in coordination relations occurred during that interval of time when the cerebellum was subjected to the action of direct current, the initial coordination relations becoming re-established on termination of cerebellar stimulation.

An intermediate group of changes evoked by cerebellar stimulation consisted either of altered intensity in the reaction of one of the muscles with its antagonist's reaction remaining unchanged, or of diminution in the reaction of one and simultaneous enhancement of the reaction in the other (Fig. 3). In other words, the direction of coordination relations was not altered completely, but only a tendency to changes in coordination relations to the reverse ones made its appearance. These intermediate changes can be regarded as transitional—forming a link between both the described types of change, since as a rule they precede the transition, observed in a number of experiments, from one type of cerebellar stimulation effect to the other. Such transition is evidently explained by a change in the functional state of the corresponding nerve centers under the influence of anesthesia or other factors associated with the experiment.

Reference to the results of cerebellar stimulation with induction current of suprathreshold strength in our own experiments and those of R. S. Mnukhina [3, 4], as well as those of Tilney and Pike [20] who extirpated the cerebellum, leads A. M. Aleksanian [1] to regard the change in coordination relations to opposite ones resulting from cerebellar effect as disturbance of the "correct functional interrelations of reflex arcs."

In our experiments the cerebellum was subjected to relatively mild influences affecting its functional state, and consequently the manifestation of its participation in the coordination of motor reactions.

Nonetheless even such mild influences not only produced changes in the intensity of motor reactions, but also reversed coordination relations, which A. M. Aleksanian regards as a disturbance of the normal cerebellar function.

However, if some changes in muscular reactions are taken to be a manifestation of the normal activity of nervous centers other changes which often occur in the same experiments and under the same conditions can hardly be regarded as a distortion of such activity.

Our experiments suggest that under normal conditions the cerebellum also not only imparts greater precision to motor reactions, "not only moderates or enhances" them, but can, under the influence of signals reaching it, even alter the established coordination relations to opposite ones without distorting but only increasing the precision of the body's motor activity.

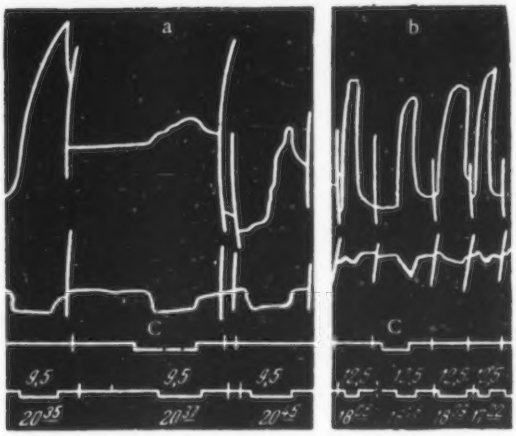

Fig. 3. Intermediate group of changes.
a) Change in the intensity of contraction of the quadriceps muscle with unchanged degree of relaxation in the semitendinosus muscle (experiment October 27, 1952); b) diminution of contraction of the quadriceps muscle and enhancement of the relaxation of the semitendinosus muscle (experiment December 23, 1952). Records the same as in Fig. 1.

During cerebellar stimulation changes in coordination relations occurred mostly by means of changes in the reaction of the extensor muscles, whereas the reaction of the flexors often remained unchanged, or, if it did change, the change was considerably less intense; this entirely agrees with literature data [7, 8, 12]. If, however, cerebellar stimulation evoked a fundamental change in coordination relations, then, in our experiments, this occurred exclusively as the result of sharp changes in flexor reactions, with only slight changes in the height of contraction of the extensor. Furthermore, even the sharpest changes in the extensor reactions did not lead to a fundamental rearrangement of coordination relations in a single experiment.

Demonstration of the role of flexor reactions in our experiments, unlike the data of other investigators, is explained by the fact that usually the effect of the cerebellum on the motor system is studied in the absence of the cerebral hemispheres or on decerebrate animals, i.e. in the presence of only the brain stem and spinal mechanisms with which is associated extensor activity which is inhibited under normal conditions by the higher divisions of the brain. Our experiments were performed on

animals with intact nervous systems, a study being made of the effect of the cerebellum on coordination relations arising on cerebral cortical stimulation.

The sharp changes in coordination relations at the expense of the flexor observed by us suggest that the cerebellum not only inhibits innate extensor reactions but also enhances flexor reactions particularly characteristic for voluntary movements. This explains the difference discovered on comparison of our experimental data with the data of A. A. Guminskii [2]. The experimental conditions in both cases are fairly similar. However, the effect of cerebellar stimulation on coordination relations was studied by A. A. Guminskii on decerebrate animals and with peripheral stimulation, whereas we worked with animals with an entirely preserved nervous system, and used stimulation of the cerebral cortex. The effects of stimulation in the two cases proved to be different. Unlike the results of our experiments, A. A. Guminskii's stimulation of the vermis and the cerebellar hemispheres produced no changes in the character of coordination relations in any of his experiments.

It would appear that when the direct interrelationship between the cerebral cortex and the cerebellum is preserved, the consistent influence of the latter on coordination relations is more complex than that which can take place in decorticate animals.

It may be supposed that under normal conditions the cerebellum not only enhances or moderates the intensity of muscular reactions but also can change the established coordination relations to opposite ones, making for greater precision of the motor reaction. In the majority of cases changes in coordination relations occurred at the expense of changes in the extensor's reaction. If, however, cerebellar stimulation evoked fundamental changes in coordination relations, these occurred at the expense of the flexor's reactions.

It seems that the cerebellum not only inhibits innate extensor reactions but also enhances flexor reactions which are particularly characteristic for voluntary movements.

SUMMARY

The author stimulated the paramedian cerebellar lobe in cats by direct current (anode or cathode). He observed 2 types of reactions of antagonist muscles to the stimulation of the corresponding motor area of the cerebral hemispheres: 1) a change in the intensity of the coordinating relations mainly due to the extensor, or 2) establishment of new antagonistic relations, mainly as a result of the altered reactions of the flexor. There was also a conversion from one type of change to another. The cerebellum intensifies the flexor reactions characteristic of voluntary movements by depressing extension.

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THE EFFECT OF SHAM FEEDING ON THE BASAL METABOLISM IN DOGS

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The physiologic mechanisms of functional connection between metabolism and digestion have not been fully investigated.

A number of experimental studies have established that changes in metabolism which arise in connection with the act of feeding cannot be explained solely by the processes of digestion and absorption of foodstuffs [4]. Data obtained by a number of authors [1, 3, 6] indicate the existence of a complex functional interdependence of gastric secretion and the level of metabolism, based on complex reflex mechanisms.

The aim of the present work has been the study of the effect of sham feeding on the basal metabolism in dogs.

EXPERIMENTAL METHOD

The experiments were performed on two dogs which had been subjected to esophagotomy, with Basov gastric fistulas. The basal metabolic rate was determined by the Douglas-Haldane method; this was done daily 15-30 minutes prior to sham feeding. Sham feeding with meat was continued until the animal refused the food offered. Experiments with sham feeding were carried out for 1 day or several days running. Two series of investigations were undertaken. In the first series sham feeding was carried out with an open gastric fistula and was accompanied by loss of gastric juice. In the second series sham feeding was carried out with a closed gastric fistula and was not associated with loss of gastric juice. Three hours after the sham feeding experiment the animal was fed in the usual way.

EXPERIMENTAL RESULTS

Relatively constant BMR values, with fluctuations of ± 6%, were obtained in control observations; these are in full agreement with literature data. The results of sham feeding experiments showed that a single sham feeding along with a loss of gastric juice evoked only slight changes in basal metabolism expressed either in a small drop in BMR or in some tendency for it to rise the next day after sham feeding.

Repeated sham feeds with an open gastric fistula which were given over a period of several days (5-9) led to a prolonged rise in basal metabolism (Fig. 1, 1). As early as the day following the first sham feed there was some rise in the BMR. Following the second sham feed the BMR reached a value equivalent to 1.68 cal·kg/hour. After the three subsequent sham feeds the BMR value dropped and reached the initial level. Two days after termination of sham feeding with an open gastric fistula there was again a rise in the basal metabolism to an average value of 1.64 cal·kg/hour. The BMR remained high during the subsequent 25 days, reaching 1.94 cal·kg/hour on some days. The oxygen utilization during this period (Fig. 1, 2) was 344 cm^3·kg/hour rising on some days to 411 cm^3·kg/hour. During the control period the average value for oxygen uptake was 277 cm^3·kg/hour. Repeated sham feeds accompanied by loss of gastric juice led, in addition to enhancement of oxidative

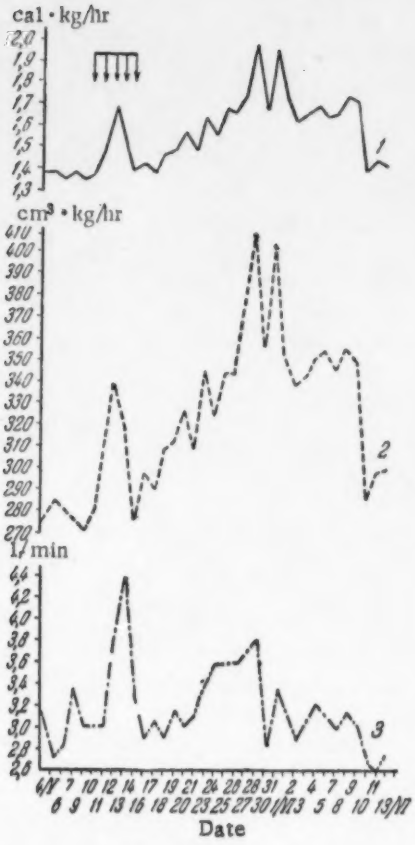

Fig. 1. Rise of BMR in dog; sham feeding with open gastric fistula accompanied by loss of gastric juice from the body. Arrows indicate days of sham feeding. 1) BMR in cal·kg/hour; 2) oxygen utilization in cm³·kg/hour; 3) pulmonary ventilation in liters/min.

processes, to increased pulmonary ventilation (Fig. 1, 3).

Subsequent repeated series of sham feeding experiments over a period of 5-9 days did not increase the changes in BMR observed in the first series of investigations. Later, during the days on which six subsequent sham feeds were given, as well as during two weeks following termination of sham feeding experiments, there was persistent increase in basal metabolism on average to 1.69 cal·kg/hour and on some days up to 1.87 cal·kg/hour. Oxygen utilization increased on average from 312 to 348 cm³·kg/hour, amounting on some days to 386 cm³·kg/hour. Increased pulmonary ventilation was also observed and was particularly marked during the three days following termination of sham feeding experiments.

In the second series of experiments sham feeding was carried out with a closed gastric fistula and was not accompanied by loss of gastric juice. The experiments showed that the first single sham feed under these conditions caused lowering of basal metabolism amounting to an average of 15% over a period of several days (1-5). In subsequent experiments with single sham feeds with a closed gastric fistula which were carried out with intervals of 1-4 weeks, the lowering of basal metabolism was less marked. Repeated sham feeds given for several (5-6) days, unaccompanied by loss of gastric juice, lowered the basal metabolism (Fig. 2, a) over a considerable period of time (up to two weeks) as can be seen from Fig. 2, a, 1. After the fourth sham feed the basal metabolism returned to the initial level. However, following the last (sixth) sham feed the value of basal metabolism again dropped. The BMR remained low (average of 1.26 cal·kg/hour) during the next 14 days, dropping on some days to 1.14 cal·kg/hour. Oxygen consumption (Fig. 2, a, 2) which was, on average, 296 cm³·kg/hour in the control period, dropped to 261 cm³·kg/hour on termination of experiments with sham feeding with closed gastric fistula, and on some days was 241 cm³·kg/hour. Pulmonary ventilation during the period of lowered basal metabolism either remained equal to the mean value observed during the control period or dropped to some degree (Fig. 2, a, 3). The respiratory coefficient varied within the range 0.69-0.9.

The data obtained were analyzed by means of experiments in which sham feeding was carried out with an open gastric fistula, but the juice secreted over a period of 3 hours was then reintroduced into the stomach (Fig. 2, b). Thus, the basal metabolism in the dog Nelka (Fig. 2, b, 1) showed an average of 1.56 cal·kg/hour during the control period. After five sham feeds with subsequent reintroduction into the stomach of the secreted juice its value dropped on average to 1.36 cal·kg/hour, and on some days was 1.20 cal·kg/hour. The basal metabolism remained low for the next 17 days. Oxygen consumption dropped on average from 322 to 278 cm³·kg/hour, and on some days it was 243 cm³·kg/hour (Fig. 2, b, 2). Some diminution of pulmonary ventilation was noted (Fig. 2, b, 3). The experiments thus demonstrated the same consistent features in the change of basal metabolism as those seen in experiments with sham feeding with a closed gastric fistula in which there was no loss of gastric juice.

Similar changes in basal metabolism associated with sham feeding under the same experimental conditions were obtained in a second dog – Kashtan.

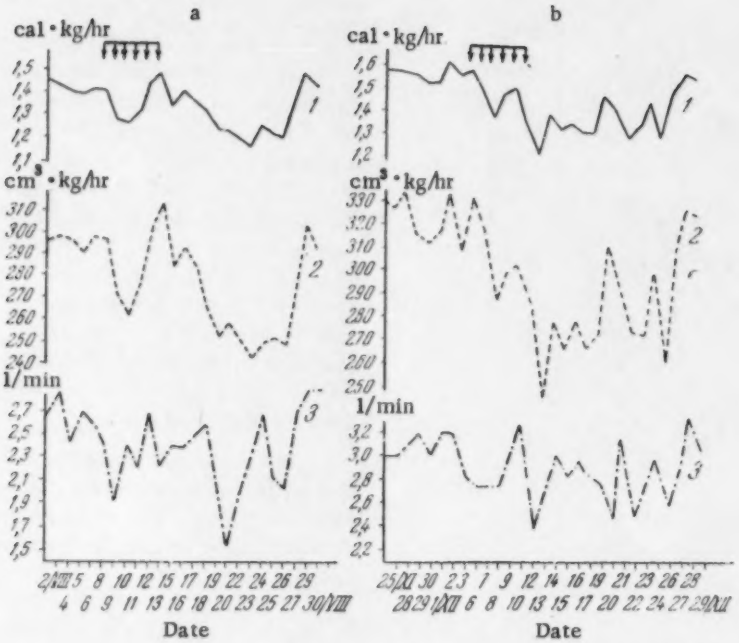

Fig. 2. Lowering of basal metabolism in dog; sham feeding with closed gastric fistula without loss of gastric juice (a); sham feeding with open gastric fistula with subsequent reintroduction into the body of secreted gastric juice (b). Arrows denote days of sham feeding. 1) Basal metabolism in cal•kg/hour; 2) oxygen utilization in cm^3•kg/hour; 3) pulmonary ventilation in liters/min.

The experimental data obtained indicate that prolonged excitation of the gastric glands in dogs associated with sham feeding continued for 5-9 successive days with open or closed gastric fistulae evokes prolonged (up to 25 days) changes in basal metabolism, the determining factor in the nature of the changes in basal metabolism being the loss or conservation of gastric juice by the body. Prolonged gastric gland secretion, not accompanied by loss of gastric juice, leads to lowering of basal metabolism, while prolonged gastric gland secretion accompanied by loss of juice leads, on the contrary, to prolonged rise in basal metabolism. The results of the present investigation agree with literature data [1, 6].

These facts again stress the close functional link between the regulation of the external secretory function of the gastric glands and basal metabolism. Changes in basal metabolism which occur on sham feeding suggest that a reflex mechanism plays the most important role in them. The prolonged character of the metabolic changes and the different directions assumed by them depending on whether the secreted gastric juice is lost or retained by the body indicate the presence of a humoral link in the regulation of the changes observed. This is also favored by literature data which point out the changes in the blood chemistry of dogs during sham feeding with an open and closed gastric fistula [5] as well as in man with relatively small losses of gastric juice [2].

SUMMARY

Reflex excitation of the gastric glands in sham feeding of dogs carried out for 5-9 days in succession with open and closed gastric fistula causes a prolonged change in the BMR (up to 25 days). Long-term secretion of the gastric glands, not connected with the loss of gastric juice, results in the decrease of the BMR. On the contrary, the loss of gastric juice by the body causes a prolonged rise in the BMR. The reflex mechanism is of the utmost importance in the changes of the BMR which appear during sham feeding. Prolonged changes in the BMR depending on whether the gastric juice which is secreted is retained or lost by the body point to a certain role played by the humoral factor in controlling these changes.

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THE POSSIBILITY OF DEVELOPMENT OF POSITIVE AND NEGATIVE CONDITIONED REFLEXES FROM SYMMETRIC AREAS OF THE SKIN

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The problems of differentiation of symmetric stimuli present considerable interest in connection with investigation of the mechanism of paired activity of the cerebral hemispheres.

There is no agreement in the literature concerning the possibility of development of positive and negative conditioned reflexes from symmetric skin areas.

Some authors consider that differentiation of stimuli from two symmetric skin areas does not take place [2], others consider that it is possible though differentiation occurs with difficulty and is fairly unstable [1, 3, 4, 5].

Detailed analysis of the positive cases of development of differentiation from symmetric skin areas as described in the literature draws attention to the fact that differentiation occurs in the presence of additional factors which potentiate the physiologic effect of cutaneous mechanical stimulation. In one case this was a marked shaking-off reflex [5] in another the use of large doses of meat-bread powder or acid for reinforcement of the tactile conditioned reflex [4, 3].

The impossibility of development of differentiation in symmetric skin areas under the usual conditions led I. P. Pavlov to suggest that the process of excitation arising on the action of a positive conditioned stimulus at a certain point of one hemisphere was constantly transmitted to a symmetric point of the other, and abolished or neutralized the process of inhibition which began to arise there in the course of development of differentiation.

From this point of view the role of additional factors is understandable: these factors, increasing the physiologic power of cutaneous-mechanical stimulation to a definite extent, favor limitation of its irradiation along the cortex, since it is known that the most readily irradiated stimuli are the weak and the very strong ones.

On the basis of these hypotheses we attempted to create those conditions, without changing the physiologic power of the cutaneous-mechanical stimulation, under which the animal would be able to differentiate symmetric stimuli.

With this aim in view we undertook to initiate simultaneously the development of a positive tactile conditioned reflex and development of differentiation from a symmetric skin area. In other words, an attempt was made to create simultaneously a focus of excitation in one hemisphere and one of inhibition in the other.

EXPERIMENTAL METHOD

Experiments were performed on 4 dogs with bilaterally exteriorized parotid glands. Observations were made on dogs with different types of nervous systems: Elza was a strong type with predominant excitatory processes, Ryzhii – a strong balanced type with good mobility of nervous processes, Volchok – strong variant of the weak type and Sedoi – weak type.*

* The typologic features of the dogs' nervous systems were determined on the basis of special tests and prolonged observation of their conditioned reflex activity.

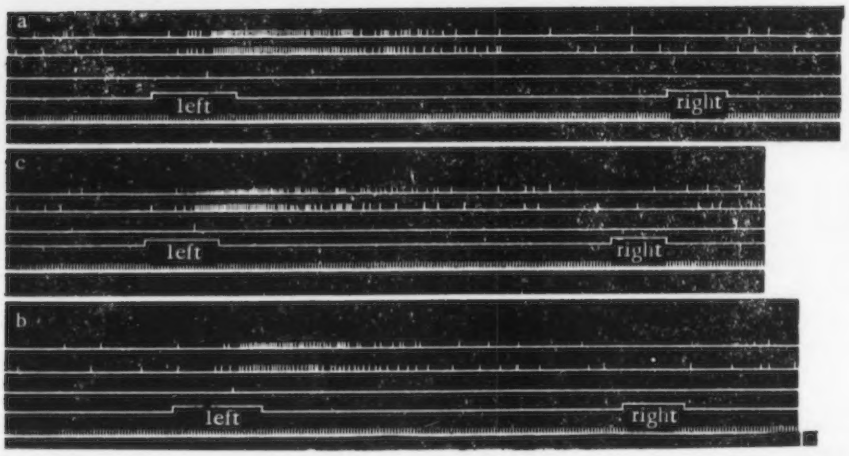

Fig. 1. Positive (left touching device) and negative (right touching device) conditioned reflexes from symmetric skin areas in the dog Elza.
a) Experiment February 5, 1957, combination No. 163; b) experiment February 7, 1957, combination No. 165; c) experiment February 25, 1957, combination No. 178. Records from above down: saliva secretion from the left gland, right gland, feed marker, conditioned stimulation marker, time marker (1 second).

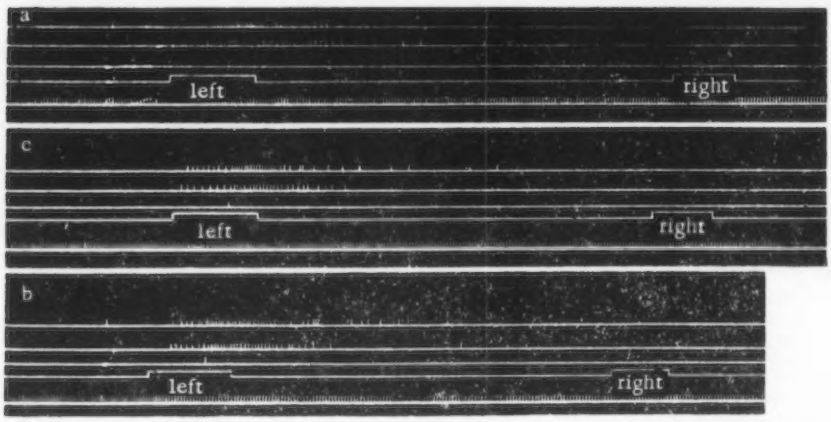

Fig. 2. Positive (left touching device) and negative (right touching device) conditioned reflexes from symmetric skin areas in the dog Ryzhii.
a) Experiment February 7, 1957, combination No. 151; b) experiment April 17, 1957, combination No. 216; c) experiment April 22, 1957, combination No. 220. Records the same as in Fig. 1.

Tactile conditioned stimuli (touching devices) were used in combination with other (auditory and light) conditioned stimuli used in the same sequence at equal intervals of time (5 minutes).

Positive conditioned reflexes were reinforced by meat-bread powder (20 g), which was slightly moistened with water.

Salivation was recorded by means of a hydraulic system according to A. I. Makarychev's method, using electric recording of drops on a film.

TABLE 1

Change in Stereotype in Experiment of March 16, 1957 on the Dog Ryzhii

Time of experiment	Ordinal no. of combinations (stim.)	Conditioned stimulus	Time of isolated action (sec)	Magnitude of cond. reflex (drops)		Latent period	Motor reaction	Food stimulus	Magnitude of uncond. reflex (drops)		Remarks
				Left gland	Right gland				Left gland	Right gland	
10 hrs 30 min	1532	Bell	20	12	9	5'5"	+	Reinforcement	49	35	
10 hrs 35 min	192	L. t. d.*	20	10	10	7'4"	+ +	Reinforcement	45	32	
10 hrs 40 min	192-a	L. t. d.*	20	4	6	15'11"	+ +	Reinforcement	44	38	Looked over in the direction of the touching device & bent low over the feed box
10 hrs 45 min	192	R. t. d.*	20	0	0	—	—	Differentiation			Sitting without change of position with back to feed box
10 hrs 50 min	734	Intermittent light	20	8	10	9'2"	+	Reinforcement	52	38	
10 hrs 55 min	1029	M120	20	9	8	9'6"	+ +	Reinforcement	53	37	
11 hrs	1533	Bell	20	12	7	8'8"	+ +	Reinforcement	36	33	

* L. t. d. = left touching device; R. t. d. = right touching device.

Negative and positive tactile stimuli from symmetric skin areas were given at the same rate – one touch per second applied to the skin on the hip.

EXPERIMENTAL RESULTS

In the case of dogs of the strong type with predominance of excitation (Elza) the introduction into the stereotype of simultaneous symmetric positive (on the left) and negative (on the right) tactile stimuli was accompanied during the first 2-3 days by a marked orientation reaction, salivation being absent during the action of both touching devices.

Later, increased saliva secretion was observed from the left and right parotid glands for several days when either the positive or negative conditioned stimulus was applied. During subsequent days a slight difference could be noted in the magnitude of conditioned reflexes depending on the action of positive or negative stimuli. However, there was fairly frequent replacement of relative differentiation by periods when conditioned reflex salivation predominated during the action of the negative stimulus. As in the case of other conditioned stimuli, the conditioned reflex salivation on the right and left was not equal. This period in the development of a positive and a negative conditioned reflex from symmetric skin areas was the longest. More or less constant adequate responses to the action of the positive and negative touching device were only observed starting from the 178th combination. Differentiation was usually incomplete (Fig. 1). This dog often showed divergence of motor and secretory reactions. An adequate motor reaction during the action of symmetric positive and negative stimuli was developed considerably earlier and was more constant.

In the case of a strong balanced type with good mobility of nervous processes (Ryzhii) salivation was absent during the action of the positive tactile stimulus during the first few days of introduction of symmetric positive and negative tactile stimuli into the stereotype, whereas during the action of the negative stimulus salivation was considerable. This was followed by a period of inhibition of the secretory reaction both during the action of the positive and the negative tactile stimulus: this period lasted several days. Then the magnitude of the positive conditioned reflexes increased gradually, differentiation became enhanced and beginning with the 133rd combination

TABLE 2

Alteration of Stereotype in Experiment of February 13, 1957 on the Dog Volchok

Time of experiment	Ordinal no. of combinations (stim.)	Conditioned stimulus	Time of isolated action (sec)	Magnitude of cond. reflex (drops)		Latent period	Motor reaction	Food stimulus	Magnitude of uncond. reflex (drops)		Remarks
				Left gland	Right gland				Left gland	Right gland	
10 hrs 30 min	1486	Bell	20	9	7	5′ 10″	+	Reinforcement	55	44	
10 hrs 35 min	169	L. t. d.*	20	5	8	6′ 2″	+	Reinforcement	38	37	
10 hrs 40 min	1005	M_{120}	20	11	14	6′ 2″	+	Reinforcement	37	42	Assumed a standing position quickly, turned and bent low over feed box
10 hrs 45 min	712	Intermittent light	20	10	12	5′ 3″	+	Reinforcement	36	36	On the 5th second turned, looked at feed box then turned away and stood sideways towards the feed box
10 hrs 50 min	169	R. t. d.*	20	2	1	8′ 19″	±	Differentiation			
10 hrs 55 min	1488	Bell	20	11	14	4′ 1″	+	Reinforcement	50	53	

* L.t.d. = left touching device; R.t.d. = righ touching device.

firm (positive and negative) conditioned reflexes from symmetric skin areas became established (Fig. 2). No divergence of motor and secretory reactions was observed. Adequate motor reaction to symmetric stimulations was formed considerably earlier than the secretory one.

In order to make certain that the formation of the conditioned reflexes occurred in fact in response to the site of action of the tactile stimulus, and not the position of the conditioned stimulus in the stereotype or the sequence of stimuli in the experiment, control experiments were staged in which the stereotype was altered. Differentiation, which was moved from third to fourth place in the experiment, did not become disinhibited but remained zero (Table 1). When the stereotype underwent more complex alteration (replacement of the negative right touching device by a metronome and shift of differentiation from third to fifth position) the dog showed reaction adequate for these stimuli – a high, positive conditioned reflex during the action of the metronome and preservation of differentiation during the action of the negative tactile stimulus, although the differentiation was somewhat weaker (1-2 drops).

The dog Volchok, a weak type (strong variant) showed slight salivation in the first 2-3 days during the action of both stimuli when symmetric positive (left touching device) and negative (right touching device) conditioned stimuli were introduced into the stereotype. Subsequently there was gradual parallel increase in positive conditioned reflexes and reduction of salivation during the action of the negative conditioned stimulus. Absolute differentiation was sometimes observed. Beginning with the 133rd combination there was formation of symmetric positive and negative conditioned reflexes from the skin. Differentiation was mostly incomplete (Fig. 3). Adequate motor reaction was formed parallelly with the secretory. At the beginning of the development of conditioned reflexes from symmetric skin areas there was divergence of the motor and secretory reactions.

Control experiments with alteration of the stereotype showed that Volchok, like Ryzhii, actually developed positive and negative conditioned reflexes at the site of action of the tactile stimulus.

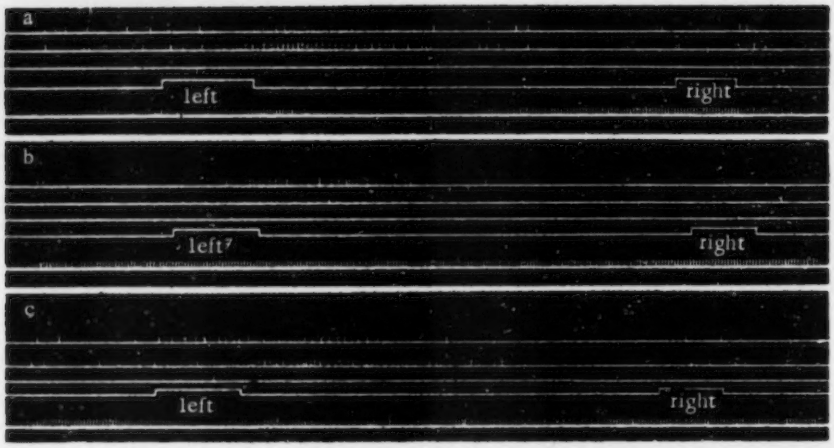

Fig. 3. Positive (left touching device) and negative (right touching device) conditioned reflexes from symmetric skin areas in the dog Volchok.
a) Experiment of February 14, 1957, combination No. 168; b) experiment of April 11, 1957, combination No. 209; c) experiment of April 22, 1957, combination No. 218. Records the same as in Figure 1.

In the case of a dog of extreme weak type (Sedoi) it proved impossible to develop stable positive and negative conditioned reflexes from symmetric skin areas despite the large number of combinations (over 300). Adequate motor reaction to the action of the positive (left) and negative (right) tactile stimuli was very marked although it was fairly inconstant. Divergence of motor and secretory reaction was observed frequently.

We thus succeeded in developing a positive and a negative conditioned reflex from symmetric skin areas in 3 dogs. Ryzhii, a dog of strong balanced type, showed replacement of a high positive conditioned reflex associated with the action of the left tactile stimulus by absolute differentiation when the right tactile stimulus was applied to a symmetric area. Two other dogs (Elza and Volchok) also developed differentiation from symmetric skin areas, although in their case it was often incomplete. Only one dog, of extremely weak type (Sedoi), proved unable to differentiate stimulation of symmetric skin areas.

It must be noted that the process of differentiation of stimulation of symmetric skin areas constitutes a fairly difficult problem for dogs. Considerable time is required for the development of a positive and a negative conditioned reflex.

The process itself is characterized by a phasic course: the reflexes tend to appear and disappear and only become more or less constant starting from a definite time different for each dog).

As shown by our experiments, the motor reaction in most cases developed earlier than the secretory one, and it was also observed in the dog of extreme weak type (Sedoi), although in this case it was less constant.

Observations on conditioned reflex regulation of the activity of paired parotid glands when unilateral symmetric stimuli were applied revealed that during the action of a positive conditioned stimulus on one side (left hip) salivation occurred from both glands but was unequal. There was predominance of activity on the part of one, then the other gland, or, if the amount secreted was equal, the onset of salivation was asynchronous on the two sides. Similar phenomena were seen when a unilateral negative stimulus was applied.

It would appear that simultaneous appearance of a focus of excitation in one hemisphere and a focus of inhibition in the other can, by means of "counterimpediment" lead to gradual limitation of the sphere of stimulation which helps the creation of the most favorable conditions for differentiation of symmetric stimuli.

SUMMARY

The possibility of development of positive and negative conditioned reflexes from symmetrical areas of

the skin was shown in experiments on dogs. However, the process of development is rather prolonged (from 133 to 178 combinations).

The rapidity and the possibility of development of symmetrical positive and negative conditioned reflexes from the skin and their stability are determined to a considerable extent by the typological peculiarities of the animal's nervous system. The author assumes that simultaneous creation of a focus of excitation in one hemisphere and that of inhibition in another is evidently of great significance for the formation of positive and negative conditioned reflexes from symmetrical areas of the skin.

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THE COMBINED ACTIVITY OF THE SPLEEN AND OTHER ORGANS

COMMUNICATION I. THE EFFECT OF EXPERIMENTAL SPLENOPATHY ON THE FUNCTIONAL STATE OF THE THYROID GLAND AND OVARIES

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Little is known of the functions of the spleen and its effect on the activity of other organs under physiologic and pathologic conditions. Relevant experimental studies and clinical observations are in many respects contradictory [2, 3, 7]. The method of organ extirpation which has yielded so much information on the function of a number of organs has not proved entirely successful with regard to the spleen; the method of administration of spleen extracts, whose specificity is yet to be proved, also has its drawbacks.

Clinical practice affords encounters both with sequelae of loss of spleen functions following splenectomy and with the influence of pathologically changed spleen on the activity of other organs and systems. It is just this latter aspect of the problem, which is of considerable practical interest, which is particularly poorly represented and developed in experimental studies owing to the lack of a suitable model of experimental splenopathy. In recent years methyl cellulose-induced splenomegaly in white rats [9, 11] has been introduced and approved as a technique which reproduces the clinical picture of Banti's syndrome in man to a satisfactory degree.

The aim of the present work has been an attempt to elucidate under experimental conditions the effect of pathologically altered spleen on the functions of other organs.

In the first series of experiments a study was made of the functional state of the thyroid in rats with experimentally induced splenomegaly. The question of correlation of thyroid function and spleen has been subjected to repeated investigation. It was noted that mice fed on thyroid gland preparations showed enlargement of the spleen. In a series of studies carried out by L. Asher et al. [4-6] antagonism between the activity of the spleen and the thyroid has been demonstrated. At the same time other authors could find no signs of increased thyroid function on morphological examination of the gland in rabbits subjected to splenectomy [8].

The present experiments were carried out on white rats weighing 110-160 g. The animals were maintained on a full diet. Splenomegaly was induced by intraperitoneal administration of 2 ml of a 2.5% solution of methyl cellulose given twice a week for 15 weeks. A noticeably enlarged spleen could be palpated in all the animals at the time of termination of the injections.

The functional state of the thyroid gland was determined by the use of radioactive iodine I^{131} which was given subcutaneously in doses of 0.6-1 microcurie at different intervals of time after the start of methyl cellulose injections. Twenty-four hours after administration of I^{131} the animals were sacrificed by exsanguination, the thyroid was excised, weighed on a torsion balance, and its radioactivity determined on a Geiger-Müller counter as percentage of the injected dose of I^{131}.

In order to discover the possible effect of methyl cellulose itself (as against the splenomegaly induced by it) on the activity of the thyroid, a group of rats was given methyl cellulose 10 days after splenectomy; in order to prevent the development of bartonellosis, these rats were given a prophylactic dose of novarsenol

(0.3 mg per 100 g body weight) at the time of splenectomy. Further control was provided by pseudosplenectomy (laparotomy without removal of the spleen) in a group of animals who were also given the same dose of novarsenol and subsequent injections of methyl cellulose.

The experimental results are given in Table 1.

As can be seen from Table 1, experimentally reproduced splenomegaly in rats leads to considerable inhibition of thyroid function as shown by the I^{131} absorption test. This effect does not occur when methyl cellulose is given to splenectomized animals (I^{131} absorption in the animals in group 2 is even somewhat increased compared to the control), which indicates that splenomegaly and not methyl cellulose itself is the leading factor in the development of depression of thyroid function. It is also indicated by the fact that diminution of isotope absorption by the gland develops in parallel with enlargement of the spleen. The main conclusion to be drawn from this series of experiments is thus the occurrence of marked depression of thyroid function under conditions of experimentally reproduced splenopathy, viz. methyl cellulose-induced splenomegaly in white rats.

The second series of experiments was devoted to the study of the functional state of the ovaries in experimentally induced splenopathy. Speculation concerning a possible connection between the spleen and the gonads was encountered as early as S. P. Botkin's lectures [1] from which the following lines are taken: "The spleen is connected with other organs: this connection cannot be determined physiologically and even less so anatomically, but it nevertheless exists. I have in mind its connection with the reproductive system."

The association of splenomegaly with hypogenitalism is not infrequently seen in clinical practice. It has been demonstrated that administration of spleen extracts to sexually immature mice leads to delay in the initial onset of estrus [12].

The view has been put forward that splenectomy in man and animals enhances the production of gonadotropic hormone by the pituitary [13]. At the same time some authors carried out experiments with extirpation of the spleen and administration of spleen extracts and found no noticeable changes in the activity of the gonads [10].

In our investigation the experiments were performed on sexually mature female white rats weighing 120-150 g. The animals were kept in individual cages on a full diet. Each rat was subjected, prior to the experiment, to determination of the character of its estrus cycle using the generally accepted technique of

TABLE 1

Effect of Experimental Splenomegaly Induced by Methyl Cellulose on I^{131} Absorption by the Thyroid in White Rats

No. of animal group	Group characteristics	No. of animals	Weight of thyroid, mg	I^{131} absorption by thyroid after 24 hrs. %
1	Intact	15	19,2±1,6	22,4±2,8
2	Splenectomy + novarsenol + methyl cellulose (15 weeks)	15	21,2±2,3	27,8±4,3
3	Pseudosplenectomy (laparotomy) + novarsenol + methyl cellulose (15 weeks)	10	23,0±2,7	7,9±1,8
4	Methyl ellulose from beginning of administration after 3 weeks	10	20,6±2,1	22,7±3,6
5	The same after 6 weeks	10	21,5±2,9	21,8±2,8
6	" " 12 "	10	23,6±2,7	12,1±3,6
7	" " 15 "	15	24,5±1,9	6,4±2,2

Note: The table shows mean values and mean quadratic deviations for each group of animals.

TABLE 2

Effect of Methyl Cellulose-Induced Splenomegaly on the Estrus Cycle in White Rats

No. of animal group	Group characteristics	No. of animals	Character of estrus cycle		
			No deviation from the norm	Moderate upset of cycle (prolonged dysestrus stage)	Cessation of estrus
1	Intact	15	15		
2	Splenectomy + novarsenol + methyl cellulose (15 weeks)	15	13	2	
3	Pseudosplenectomy + novarsenol + methyl cellulose (15 weeks)	10		1	9
4	Methyl cellulose from beginning of administration after 3 weeks	25	25		
5	The same after 6 wks.		22	3	
6	" " " 12 "			19	6
7	" " " 15 "			4	21

TABLE 3

Effect of Methyl Cellulose-Induced Splenomegaly on the Gonadotropic Hormone Content of the Hypophysis in White Rats

No. of rat group	Group characteristics	Dose of rat	Number of test mice	Reaction of mice		
				Enlargement of uterus and ovaries	Estrus	Corpora lutea
	Intact	4	6	2	1	1
		5	6	6	6	5
	Experimentally induced splenomegaly (methyl cellulose, 15 weeks)	3	6	-	-	-
		4	6	1	1	-
		5	6	6	6	6

vaginal smears; this was done over a period of 3 weeks. Then the experimental group of rats was given methyl cellulose as described above for a period of 15 weeks and the character of the estrus cycle was studied. In some rats the spleen was removed 10 days prior to beginning administration of methyl cellulose and a prophylactic injection of novarsenol was given; in another group of rats used as controls pseudosplenectomy (laparotomy), with novarsenol administration and subsequent injections of methyl cellulose was carried out.

The results of the experiments are presented in Table 2.

It follows from Table 2 that administration of methyl cellulose leads gradually to ever-more-noticeable disturbance of the estrus cycle up to complete cessation of estrus in an overwhelming majority of the animals 15 weeks after the beginning of injections. Methyl cellulose does not produce this effect in animals with removed spleen, which indicates that splenomegaly plays the leading role in the development of the phenomenon in question.

The third series of experiments was staged in order to discover more precisely the mechanism of the effect of methyl cellulose-induced splenopathy on the estrus cycle.

First of all a study was made of the reactivity of the vaginal mucosa to estrogenic influence in rats with splenomegaly. For this purpose 5 rats with splenomegaly produced by 15 weeks of methyl cellulose injections and cessation of estrus were given subcutaneously 10 international units folliculin each. After 72-96 hours cornification of the epithelial cells characteristic for the estrus phase was noted in all 5 rats.

Hence the reactivity of the vaginal mucosa is preserved in rats with splenomegaly.

A fourth series of experiments was then carried out to investigate the content of gonadotropic hormones in the hypophysis of rats with splenomegaly.

Hypophyseal hormone was tested on infantile mice weighing 6-8 g. The rat pituitaries were excised, weighed and triturated with physiologic solution until a fine suspension was formed; this was injected into infantile mice. After 100 hours the state of the reproductive apparatus was examined (the mice were given hypophyseal suspension five times in accordance with the accepted technique of gonadotropic hormone standardization).

The results are given in Table 3.

Table 3 shows that 1 international unit of gonadotropic hormones constitutes 5 mg hypophyseal substance both in the control animals and in rats with splenomegaly. (The criteria for judging the presence in the hypophyseal suspension of follicular stimulating hormone were the appearance of estrus in mice and increase in the weight of the uterus and ovaries, while the criterion for the presence of luteinizing hormone was demonstration of corpora lutea.) There is thus no change in the gonadotropic hormone content of the pituitary in rats with experimental splenopathy. Consequently, by the process of exclusion, it may be supposed that the cause of estrus cycle disturbance observed in rats with methyl cellulose-induced splenomegaly lies in ovarian dysfunction. In order to check this hypothesis we administered subcutaneously 20 international units of prolan to 8 rats with splenomegaly; not a single rat reacted to prolan injection by the appearance of estrus, signifying the loss of ovarian sensitivity to gonadotropic hormone. All these experiments indicate that experimentally induced (methyl cellulose) splenopathy is associated with definite depression of thyroid and ovarian activity.

SUMMARY

The author studied the effect of experimental splenomegaly (caused by the intraperitoneal injection of methyl cellulose to white rats) on the functional state of the thyroid gland (by the absorption of I^{131} by the gland) and of the ovaries (by studying the estrus cycle). It was established that splenopathy brought about pronounced depression of the functional activity of the thyroid gland and the ovaries. This phenomenon is caused by splenomegaly since the administration of methyl cellulose to splenectomized animals does not cause such changes in the thyroid gland and the ovaries. It was demonstrated that the changes in the estral cycle in splenopathy did not depend on disturbance of the gonadotropic activity of the hypophysis or on changes in the sensitivity of the vaginal mucosa to the estrogenic action.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CONCERNING THE REFLEX MECHANISM OF ANTIBODY FORMATION

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The question concerning reflex formation of antibodies without circulating antigen, first posed by R. Pfeiffer [14] and then by E. Friedberger [9-12] is at present being elaborated by A. N. Gordienko [2].

The present work introduces experimental material obtained during the study of the processes of antibody formation under conditions of antigen action on chemoreceptors of the isolated carotid sinus in rabbits (75 experiments). The data presented below constitute the results of verification of A. N. Gordienko's experiments but, unlike Gordienko who ligated all the vessels and tissues of the carotid sinus (excluding the sinocarotid nerve) en masse with one common ligature, we carried out very careful, conservative preparation of the blood vessels in the area of the bifurcation of the common carotid artery, aiming not to impair circulation in the carotid glomus and nerve. It is known that thrombosis of glomus vessels leads to loss of excitability of its chemoreceptors [1, 13, etc.). Our method of isolation allows the chemoreceptors to preserve their functional properties, i.e., excitability in response to physiologically adequate stimuli, and therefore for the sake of brevity this method will henceforth be referred to as "physiologic."

EXPERIMENTAL METHODS

Under light ether anesthesia a single thick ligature is passed under the common carotid cephalad to the thyroid artery; another such ligature is passed under the external carotid artery at the site of branching of the lingual artery. By means of these 2 ligatures an assistant manipulates the area of the bifurcation upwards and medially. The internal carotid, occipital, superior pharyngeal, lingual and other small (2-3) branches are exposed in this position. All the vessels, including the common carotid and the external carotid arteries are tied with 2 ligatures. To prevent accidental access of antigen to the surface of the wound the latter is packed with sterile tampons. Antigen (dysentery vaccine, complete typhoid or dysentery antigen) is introduced into the sinus through a puncture in the common carotid artery; the amount used is from 0.06 to 0.1 ml. At the moment of withdrawal of the syringe a third ligature is tightened around the common carotid artery, this ligature being the most cephalad. After the injection the blind sac formed by the ligature of the vessels in the sinus area should show noticeable distension. The tampons are carefully replaced by fresh ones. The wound is covered with a dry tampon and left for 5 minutes after which all the vessels are divided between the ligatures, the sinocarotid nerve being the last to be sectioned, as far from the sinus as possible, and the carotid sinus is then excised. The wound is closed in two layers. Blood for antigen titer is withdrawn from the pinna vein or from the heart 15 minutes, 1 hour, 2 hours and 24 hours after the injection of antigen into the sinus. The presence of antibodies in the blood is determined on the 4th, 7th, 10th and 14th day following antigen introduction.

The sinocarotid nerve is dissected away from the carotid sinus removed from the body. The sinus is cut up with scissors into 1 ml sterile physiologic solution, "extracted" in the refrigerator for 18-20 hours and the amount of antigen in the "extract" determined. The sinocarotid nerve, separated from the sinus, is treated similarly. Antigen is determined by means of complement fixation reaction in the cold.

Reaction ingredients: antigen (being sought) – blood serum, "extracts"; antibody – precipitating typhoid or dysentery serum (precipitin titer –1:800,000, complement fixation (cold) titer –1:1,000,000) prepared at the I. I. Mechnikov Institute of Vaccines and Sera (typhoid serum series 85, dysentery series 147); complement-desiccated complement prepared at the same Institute (series 98-105), hemolytic serum prepared at the same

TABLE 1

Control Experiments

No. of rabbit	Experimental conditions	Antigen in the blood		Agglutinins in the blood	
		Initial titer	Maximal titer	Initial titer	Maximal titer
7	A. Saline introduced into sinus and sinus removed 5 min later	0	0	1/10	1/10
8	The same	0	0	1/10	1/20
53	" "	0	0	1/20	1/20
54	" "	0	0	1/5	1/10
5	B. Saline introduced into sinus and sinus removed; heated dysentery vaccine (2,000,000 bodies) given intravenously	0	1/2	1/20	1/640
6	The same	0	Trace	1/10	1/960
9	" "	0	»	1/10	1/800
10	" "	0	»	1/30	1/500
11	" "	0	1/2	1/10	1/300
12	" "	0	1/2	1/20	1/600
1	C. Dysentery vaccine (2,000,000 bodies) given intravenously	0	Trace	1/5	1/320
2	The same	0	»	1/10	1/960
3	" "	0	1/2	1/10	1/480
4	" "	0	1/2	1/5	1/640
39	" "	0	Trace	1/20	1/1000
40	" "	0	1/2	1/10	1/400
25	D. Complete dysentery antigen (0,01 mg) given intravenously	0	1/4	1/20	1/1000
26	The same	0	1/4	1/10	1/4000
27	" "	0	1/2	1/20	1/2000
28	" "	0	1/2	1/10	1/1200

Institute (series 14, titer 1:1600), sheep erythrocytes 3% suspension prepared ex tempore by the usual method.

The reaction was carried out in the refrigerator at 2° over a period of 18-20 hours, the results being read after allowing the material to stand in a thermostat at 37-38° for 25-30 minutes.

Blood antibodies were determined by agglutination reaction.

EXPERIMENTAL RESULTS

First series – control experiments. Preliminary control experiments were carried out in order to discover the following: the effect of operative removal of the carotid sinus on the initial antibody titer (4 experiments); effect of operative removal of the carotid sinus on the process of antibody formation on intravenous injection of antigen (6 experiments); dynamics of antibody formation on intravenous injection of small amounts of dysentery antigens (10 experiments). The amount of antigen for intravenous injection was chosen in accordance with our supposition as to the quantity that could pass from the isolated sinus into the blood if such a possibility existed.

The results of the experiments are presented in Table 1.

Second Series – investigation of antibody formation under conditions of "physiologic" isolation of the carotid sinus. "Physiologic" isolation of the carotid sinus differs favorably from the method proposed by A. N. Gordienko in that it preserves the excitability of the carotid glomus receptors. Absolute isolation, according to A. N. Gordienko, can only be achieved with loss of sensitivity of chemoreceptors as the result either of thrombosis of vessels supplying the glomus or by ligation of these vessels. Since it is absolutely essential to preserve receptor

TABLE 2

Introduction of Antigens into the Isolated Carotid Sinus

No. of rabbit	Experimental conditions	Antigen in the blood		Agglutinins in the blood	
		Initial titer	Maximal titer	Initial titer	Maximal titer
13	A. Heated dysentery vaccine ($2 \cdot 10^8$ bodies)	0	Trace	1/20	1/320
14	The same	0	»	1/10	1/640
15	» »	0	»	1/10	1/480
16	» »	0	0	1/10	1/20
19	» »	0	Trace	1/20	1/300
20	» »	0	»	1/5	1/800
21	» »	0	0	1/20	1/10
22	» »	0	Trace	1/10	1/400
23	» »	0	Not clear	1/10	1/800
24	» »	0	» »	1/20	1/300
29	» »	0	Trace	1/30	1/500
30	» »	0	»	1/20	1/600
31	» »	0	»	1/10	1/800
35	» »	0	0	1/10	1/10
36	» »	0	Trace	1/10	1/600
37	B. Complete dysentery antigen (0.06-0.1 mg)	0	»	1/10	1/800
38	The same	0	1/2	1/20	1/500
41	» »	0	Trace	1/10	1/1200
42	» »	0	»	1/10	1/600
43	» »	0	»	1/10	1/1800
48	C. Complete typhoid antigen (0.06-0.1 mg)	0	»	0	1/320
49	The same	0	0	1/5	1/20
50	» »	0	1/2	0	1/5000
51	» »	0	Trace	0	1/1000
52	» »	0	»	1/5	1/5000
57	» »	0	1/2	0	1/1200
58	» »	0	Trace	0	1/600
59	» »	0	»	1/5	1/1000
60	» »	0	0	1/10	1/20
61	» »	0	Trace	0	1/400
17	D. Dysentery vaccine introduced after ligation of sinocarotid nerve	0	0	1/30	1/30
18	The same	0	0	1/10	1/20
32	» »	0	0	1/10	1/10
33	» »	0	0	1/20	1/10
34	» »	0	0	1/5	1/10
62	E. Complete typhoid antigen (0.06-0.1 mg) administered after ligation of sinocarotid nerve	0	0	0	0
63	The same	0	0	0	0
64	» »	0	0	1/5	1/10
65	» »	0	0	0	0
66	» »	0	0	1/10	0
67	» »	0	0	1/5	1/20
68	» »	0	0	0	0

excitability to antigen in order to prove the reflex nature of antibody formation, we made use of "physiologic" isolation.

The following experiments were undertaken in this series:

1) introduction of 0.06-0.1 ml heated dysentery vaccine into the isolated carotid sinus (15 experiments);

2) introduction of 0.06-0.1 mg complete dysentery (5 experiments) or typhoid (10 experiments) antigen;

3) introduction, for control purposes, of the corresponding antigens into the isolated carotid sinus after its denervation; denervation was effected by tying the sinocarotid nerve with two ligatures and subsequent division (12 experiments).

The experimental results are presented in Table 2.

As can be seen from Table 2, 25 out of the 30 rabbits taken for the main experiments showed minimal amounts of antigen in the blood following introduction of the antigen into the physiologically isolated carotid sinus. The appearance of antigen in the blood was accompanied by subsequent rise in antibody titer.

We wish to draw particular attention to the results of 5 experiments (rabbits No. 16, 21, 35, 49 and 60); complete isolation of the carotid sinus occurred in the course of these experiments (as the result of thrombosis of glomus capillaries, most probably) as proved by absence of antibody from the blood, shown by repeated search for it by the cold complement fixation method. Absolute isolation of the carotid sinus area from blood and lymph circulation of the body as a whole thus completely excludes the possibility of reflex antibody formation under the influence of antigen action on glomus chemoreceptors. Antibodies are only formed when the antigen passes from the sinus into the blood; in those cases in which no antigen is found in the blood there is also no antibody formation.

What is the manner in which antigen passes from "isolated" sinus into the blood? A 3rd series of experiments was undertaken in order to answer this question.

Third series – resorption of antigen from the isolated carotid sinus. 0.06-0.1 mg complete typhoid antigen in 1:1000 dilution was introduced into the isolated carotid sinus. Contact with the antigen lasted for 5 minutes

TABLE 3

Antigen Resorption from the Isolated Carotid Sinus

No. of rabbit	Time of sinus–carotid contact, min.	Amt of antigen introduced into sinus, mg	Amt. of antigen detected			
			In the sinus		In nerve γ	In blood γ
			mg	% of introduced		
50	5	0,1	0,09	90	4	6
51	5	0,1	0,08	80	2	18
52	5	0,08	0,06	75	2	18
57	5	0,09	0,06	67	4	26
58	5	0,06	0,05	84	4	6
59	5	0,08	0,06	75	2	18
60	5	0,06	0,05	84	0	10
61	5	0,10	0,06	60	Trace	40
69	15	0,06	0,03	50	2	28
70	15	0,1	0,06	60	Trace	40
71	15	0,1	0,07	70	1,5	28,5
74	15	0,08	0,05	63	4	26
75	15	0,1	0,06	60	2	38
72	30	0,1	0,04	40	3	57
73	30	0,1	0,05	50	2	48
76	30	0,08	0,025	32	2	73
77	30	0,1	0,05	50	1,5	48,5
78	30	0,08	0,03	38	Trace	50

(8 experiments), 15 minutes (5 experiments) and 30 minutes (5 experiments). The excised sinus and sinocarotid nerve were then extracted and their antigen content determined.

The results of these experiments are given in Table 3.

It follows from Table 3 that after 5 minutes' contact 60-90% of the administered amount of antigen remains in the isolated sinus (10-40% antigen is resorbed); after 15 minutes' contact 50-70% antigen remains (30-50% is resorbed) and after 30 minutes' contact only 32-50% of the introduced antigen remains in the isolated sinus. Therefore as early as the first 5 minutes a considerable amount of the introduced substance is resorbed from the isolated carotid sinus. The speed of resorption during the first 5 minutes is enhanced by increased pressure within the sinus; during the subsequent 15-30 minutes resorption continues, but less intensely, owing to lowering of pressure within the sinus.

Similar results were obtained by Iu. V. Sergeev in I. A. Oivin's laboratory; he showed that half the radioactive isotope (NaI^{131}) introduced into the isolated carotid sinus (rabbit) was resorbed in 29 minutes on average.

Parallel determination of antigen content in the sinocarotid nerve and the cellular tissue around it showed that it contained small amounts of antigen which could nevertheless be determined by the cold complement fixation method; these amounts varied from traces to 4 γ. The amount of antigen in the nerve does not depend substantially on the duration of resorption; consequently in this case the perineural lymphatic and hematogenic pathways of the nerve serve as a peculiar "cable" along which the antigen leaks from the isolated sinus into the blood.

The difference (leakage of antigen from the sinus – antigen in the sinocarotid nerve) could furnish data for determination of the amount of antigen resorbed into the blood. It amounted to 6-40 γ after 5 minutes.

The number of antigen molecules contained in 6 γ can be determined on the basis of Avogadro's number. The number of molecules in 1 g/mol (Avogadro's number) is equal to $6.023 \cdot 10^{23}$; 1 g/mol antigen equals 1,000,000 g, therefore 1,000,000 g antigen contains $6.023 \cdot 10^{23}$ molecules, 1 g antigen contains $6.023 \cdot 10^{17}$ molecules, 1 γ antigen contains $6.023 \cdot 10^{11}$ molecules, and 6 γ antigen contains $36.138 \cdot 10^{11}$ molecules.

It is known that each molecule of antigen (diphtheria toxoid) introduced into the body is associated with the formation of a minimum of 1,000,000 molecules of antibody within 3 weeks [7]. Clearly then the passage of such a seemingly small amount of antigen as 6 γ from the sinus into the blood is more than sufficient for successful sensitization of the animal organism.

We conclude on the basis of our experiments and in agreement with the conclusions of P. F. Zdrodovskii, I. A. Oivin, D. F. Pletsityi and many others that experiments on the isolated carotid sinus do not give convincing proof of the reflex mechanism of antibody production. A. N. Gordienko's assertion that the carotid sinus receptors participate in the mechanism of antibody production results from the erroneous methods used by him and his collaborators in their work on the study of the reflex mechanism of antibody production.

SUMMARY

It was demonstrated in this work that the complete isolation of the carotid sinus from the blood and the lymph circulation excludes the possibility of the reflex production of antibodies when the antigen acts on the glomus chemoreceptors alone. It was established by investigation of antigen resorption from the isolated carotid sinus by the leakage determination method that on the average about 24% of the antigen injected into the sinus is resorbed within the first 5 minutes. The determination of the antigen content in the sinocarotid nerve under these conditions demonstrated that one of the important paths of resorption was the perineural lymphatic spaces, and possibly the blood capillaries.

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THE EFFECT OF ADRENOCORTICOTROPIC HORMONE ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

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Atherosclerosis is one of the most important problems of modern medicine, and is at present regarded as an independent disease of the vessels, not connected with the changes caused in them by age. The leading role in the complex pathogenesis of this disease is ascribed to primary disturbances of lipoid metabolism [1, 2, 3, 4, 5, 9, 10, 14, 19]. The modern infiltration theory of atherosclerosis proposed by N. N. Anichkov is shared by many American investigators of this problem [25, 29, 30, 32].

Thanks to the classic experiments of N. N. Anichkov and S. S. Khalatov, research workers have received a convenient biologic model with whose help many questions of morphogenesis, pathogenesis, therapy and prophylaxis of atherosclerosis have been clarified. The essence and the cause of the disturbances of lipoid-cholesterol metabolism which lead to atherosclerosis have not yet been finally elucidated. In recent years a number of Soviet and foreign scientists have established the effect of some vitamins, neurogenic medicinal preparations, and hormones on this process [4, 13, 15, 16, 17, 24, 27, 30, 31]. Evidently the main significance in the development of atherosclerosis belongs to disturbances of neuroendocrine regulation, chiefly of lipoid metabolism. The data on the effect of adrenocorticotropic hormone of the pituitary (ACTH) and adrenal cortical hormones (cortisone, hydrocortisone, desoxycorticosterone) on cholesterolemia and atherosclerosis are conflicting. Some authors [22, 23, 28] note an inhibitory effect of these hormones on the development of experimental atherosclerosis, others either find no definite effect [24, 25, 30] or find exacerbation of atherosclerosis [21, 26, 32]. Dury (USA) reported at the IV Congress of Gerontologists that experimental atherosclerosis was ameliorated under the influence of cortisone; he associated this with a decreased permeability of blood vessel walls to cholesterol.

Taking into account the recent widespread use of adrenal cortical hormones and their stimulator ACTH for the treatment of various diseases and the important part played by these hormones in the regulation of lipoid metabolism, as well as the conflicting nature of literature data on this question, we undertook to study the effect of the Soviet ACTH preparation on experimental and clinical atherosclerosis [7].

EXPERIMENTAL METHOD

Two series of experiments on 40 rabbits weighing about 3 kg were staged during 1956-7 in order to study the effect of ACTH on atherosclerosis. The work was carried out at the S. I. Chechulin Central Scientific-Research Laboratory at the I Moscow Order of Lenin Medical Institute. All the animals were divided into control and experimental groups. In the first series of experiments the control animals were given cholesterol 0.1 g per 1 kg body weight for a period of 65 days, in the second series the dose was 0.2 g per 1 kg body weight, continued for an average of 105 days. The experimental group received, in addition to 10% solution of cholesterol in sunflower seed oil, subcutaneous injection of ACTH in doses of 5-10 units (up to 380 units over the experimental period) in the first series and 10-15 units starting from the 30th day of cholesterol feeding in the second series (total 600-900 units per rabbit).

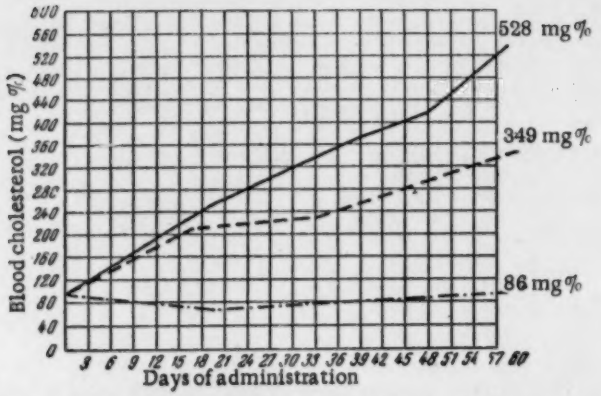

Fig. 1. Effect of ACTH on experimental hypercholesterolemia (first series of experiments).
Records: —— blood cholesterol level in rabbits given cholesterol; ---the same on administration of cholesterol + ACTH; ---the same on administration of ACTH alone.

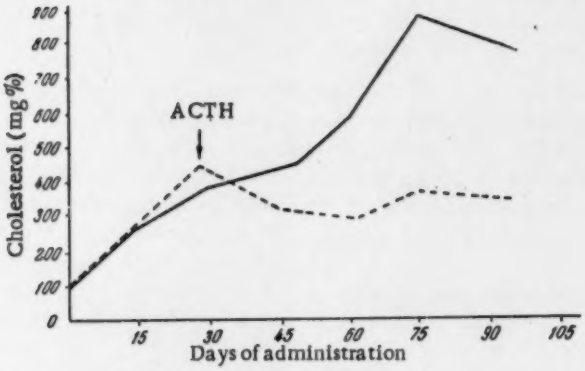

Fig. 2. Effect of ACTH on experimental hypercholesterolemia (second series of experiments).
Records: —— cholesterolemia in control animals (cholesterol); ---the same in experimental rabbits (cholesterol + ACTH).

In the first series of experiments 7 animals received only injections of ACTH without cholesterol feeding. All the animals were subjected to blood cholesterol determinations by Grigaut's method prior to beginning the experiment.

Analysis of the data obtained shows that hypercholesterolemia in the experimental animals was considerably lower and developed more slowly than in the case of the control animals (Figs. 1, 2). The control animals had a hypercholesterolemia of 500-800 mg% by the end of the experiment, whereas in the experimental animals it was 350-360 mg%. Hypercholesterolemia in the second series of experiments began to fall from the moment of ACTH administration (Fig. 2). The blood cholesterol level was checked in 5 rabbits 18-20 hours after the first injection of 15 units ACTH, and in all cases there was marked decrease of hypercholesterolemia (by 50-250 mg%).

In the case of rabbits which received ACTH alone the cholesterolemia remained practically unchanged and was within normal limits. At the end of the experiment the rabbits were sacrificed by the air embolus

method; the heart and aorta were excised in toto and examined for lipoidosis by means of Sudan III staining, also in toto.

EXPERIMENTAL RESULTS

The aortas of the control group showed marked atherosclerotic changes, mostly along the whole length of the aorta, often with involvement of the area at the mouth of the coronary vessels. In some preparations from the second series of experiments lipoidosis spread within the coronary arteries. Considerable lipoidosis was also observed in the pulmonary artery.

The degree and spread of aortic lipoidosis in the experimental group of rabbits (cholesterol + ACTH) were less pronounced than in the control group (Fig. 3, a, b). Less marked lipoidosis was found in the experimental group in the pulmonary artery and in the region of the mouth of the main coronary vessels.

There were no aortic changes in the rabbits who had received ACTH alone.

The data obtained point to an inhibitory weakening action of ACTH on experimental atherosclerosis which is evidently associated with its inhibitory effect on the development of hypercholesterolemia and lowering of blood vessel wall permeability observed on administration of ACTH.

It is possible that in our experiments the inhibitory effect of ACTH on the development of alimentary hypercholesterolemia is connected with increased breakdown of excess cholesterol, which in some measure compensates for the metabolic disturbance. Such a supposition agrees with the data of S. M. Leites [11, 12] concerning the regulatory effect of ACTH in the process of compensation for some deviations in metabolism (adaptational action of ACTH). The constantly administered large doses of excess cholesterol in our experiments naturally prevented complete normalization or compensation of cholesterol metabolism.

It should be added that in some of the rabbits in the experimental group in the second series of experiments determinations of arterial blood pressure were made on the carotid artery exteriorized in a skin flap; the cuff method was used. The arterial pressure was increased against the background of ACTH administration and varied from 110 to 170 mm Hg. Despite this there was no exacerbation of atherosclerosis in the experimental animals. This is apparently connected with the influence of ACTH on factors which inhibit atherosclerosis, being more pronounced than the unfavorable effect of hypertension on atherosclerosis.

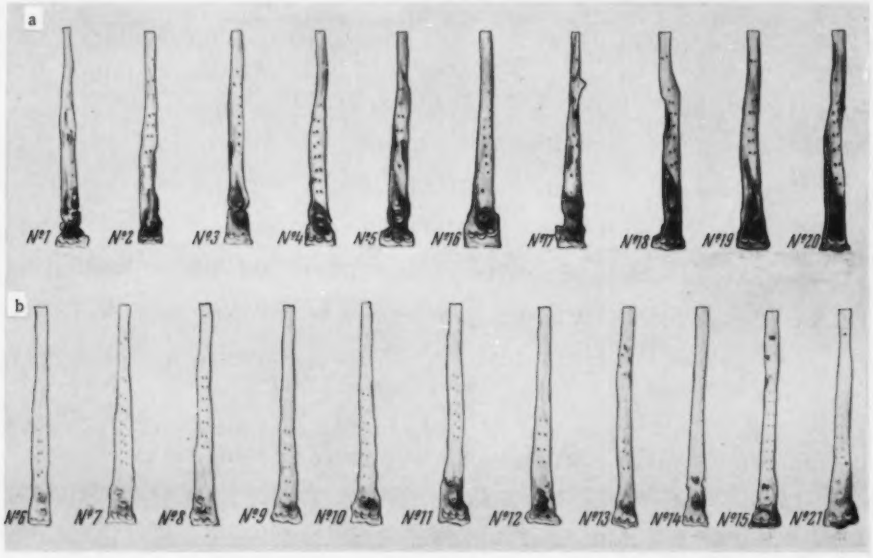

Fig. 3. Rabbit aortas (second series of experiments).
a) Control group, given cholesterol; b) experimental group given ACTH + cholesterol.

Since these experiments made use of a hormone which stimulated the adrenal cortex, we were interested to see what changes took place in this gland. The adrenals of the rabbits which had received cholesterol were considerably enlarged in size and their weight (1.38-1.45 g) was several times that of normal adrenals. Such enlargement is explained by lipoid infiltration of the adrenal cortex.

The adrenals of rabbits which had received cholesterol and ACTH showed less fatty infiltration but were also markedly enlarged due to hyperplasia of the secretory zone of the adrenal cortex, associated with its increased function.

The following changes were found in the adrenals of rabbits which had received ACTH alone: increase in size, weight (up to 0.88 g), hyperplasia of the fascicular and glomerular zones of the cortex, unequal distribution of lipoids and their reduced amount compared with normal adrenals. All these changes are characteristic for a state of increased function of adrenal cortex [6].

According to literature data [18, 20] prolonged administration of ACTH to rabbits is accompanied by secretion into the blood not only of large amounts of corticosterone but also of hydrocortisone.

It must be noted that despite the sufficiently clear and convincing nature of the results obtained concerning the effect of ACTH on the development of experimental atherosclerosis and hypercholesterolemia in man, we are far from suggesting that this preparation be used as a therapeutic agent for atherosclerosis in man, since ACTH has a number of other undesirable side effects on the organism. However, the present work can have definite meaning in defining indications and contraindications for the therapeutic use of ACTH. It is possible that disturbances in cholesterol metabolism are not in themselves contraindications for the use of ACTH since the experimental data obtained point to a regulatory effect of ACTH in disturbances of cholesterol metabolism.

SUMMARY

Two series of experiments were carried out on 40 rabbits. The author studied the effect of ACTH on the development of experimental atherosclerosis (according to N. N. Anichkov's method). The data obtained make it possible to draw a conclusion on the inhibiting effect of ACTH in the development of hypercholesterolemia and experimental atherosclerosis.

Prolonged administration of ACTH increases the functional activity of the adrenal gland, while the arterial pressure shows a certain rise.

Single administration of ACTH in presence of hypercholesterolemia decreases the blood cholesterol level in 18-20 hours. It is probable that ACTH has a controlling effect on the disturbed cholesterol metabolism.

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THE EFFECT OF PHYSICAL STRAIN ON THE DEVELOPMENT OF EXPERIMENTALLY INDUCED ATHEROSCLEROSIS

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The effect of physical exertion on the development of atherosclerosis has not yet been studied systematically. Observations reported in the literature show that atherosclerosis is less frequent and less pronounced in persons who do physical work and lead active lives than in persons who do intellectual work and lead sedentary lives; the coronary arteries of persons in the latter group are particularly likely to be affected. Ia. L. Rappoport [2] found severe atherosclerosis to be 3 times more common in persons doing intellectual work than in persons doing physical work.

We found no reports in the literature of experimental investigation of this matter and therefore determined to study the effect of physical exertion on the rate of development, the degree, and the character of atherosclerosis of the aorta and coronary arteries, using N. N. Anichkov's cholesterol-feeding technique for inducing atherosclerosis.

EXPERIMENTAL METHODS

Our experiments were performed on 45 rabbits of the same breed which had been kept under identical conditions and fed a standard diet; each rabbit weighed from 2000 to 2500 g. We induced atherosclerosis of the aorta and coronary arteries by feeding the rabbits a 10% solution of cholesterol in vegetable oil daily for six months (this corresponded to 0.3 g of cholesterol per kg of body weight).

For physical exertion the rabbits were made to run daily on an electrically driven wheel during the whole course of the experiment. After each cholesterol feeding rabbits were made to run on the wheel until they began to become exhausted. This usually required from 5 to 7 minutes of running, with the wheel making 12 revolutions per minute; since the inner circumference of the wheel was 4 m 75 cm, the rabbits ran 57 m per minute.

The experimental animals were divided into 3 groups. The rabbits of group I, the control group (12 rabbits), were fed cholesterol but were not made to exercise. The rabbits of group II (25 rabbits) were fed cholesterol and were also made to exercise. The rabbits of group III (8 rabbits) were made to exercise but were not fed cholesterol.

Twice a month we measured the blood cholesterol of all the rabbits, using Grigaut's method. At the beginning and at the end of the experiment the activity of the thyroid gland of 25 rabbits (10 from group I, 15 from group II) was studied by means of radioactive iodine, I^{131}. At the end of the experiment all the animals were killed by the injection of air into an ear vein. The aorta and heart were removed from each animal. The aorta was stained in toto with Sudan III. The heart was studied in serial sections in the usual way. We indicated the degree of atherosclerosis of the aorta and coronary arteries which we found with plus marks: + slight, ++ moderate, +++ pronounced alterations.

EXPERIMENTAL RESULTS

Twelve animals died in the course of the experiment: 1 rabbit of group I (of pneumonia), and 11 rabbits of group II. These animals died during the 3rd, 4th, and 5th months of the experiment. Electrocardiographic examinations showed that before their death the animals were suffering from acute coronary insufficiency. Histological study of the heart disclosed extensive areas of necrosis in the muscle of the left and, less often, of the right ventricles; in one case an acute aneurysm of the heart was found in a necrotic area.

The rabbits of group II died at different times during the period of cholesterol feeding (during the 3rd, 4th, and 5th months), but in order to compare the degree of atherosclerosis which developed in the rabbits of group II with that which developed in the rabbits of group I it was necessary to study all animals which had been fed cholesterol for 6 months. In this report we will consider only the 14 rabbits (out of the original 24) of group II which did not die during the experiment but which were killed at the end of 6 months (our study of the myocardium of all the rabbits used in the experiment is described in a separate report).

In group I (rabbits which were fed cholesterol but not made to exercise) the blood cholesterol rose quickly to high values, to as much as 1730 mg % in individual cases, and to an average of 1028 mg %; afterwards it fell gradually to an average of 627 mg % at the end of the experiment.

In group II (rabbits which were fed cholesterol and also made to exercise) the blood cholesterol was considerably lower than that of the rabbits of group I; in most cases it did not exceed 600-700 mg % (Fig. 1), toward the end of the experiment it fell to an average of 310 mg %. The blood cholesterol of the rabbits in group III (rabbits which were not fed cholesterol) varied within normal limits.

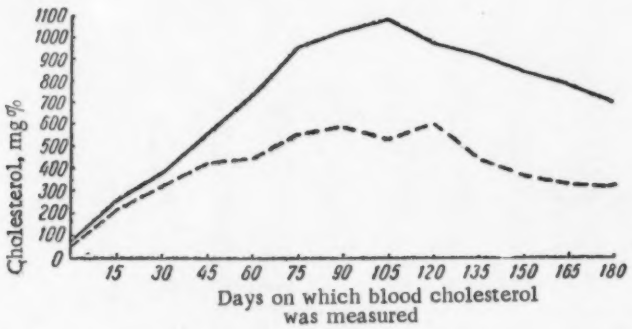

Fig. 1. The effect of physical exertion on the development of hypercholesterolemia.
---Cholesterol and exercise; ——cholesterol only.

TABLE 1

Changes in Blood Cholesterol Following Physical Exertion

Nos. of the rabbits of Group II	Cholesterol in mg %			Nos. of Group III rabbits	Cholesterol in mg %			Nos. of healthy rabbits	Cholesterol in mg %	
	before exercise	immediately after exercise	3 hr. after exercise		before exercise	immediately after exercise	3 hr. after exercise		before being placed on wheel	after being placed on wheel
165	218	280	210	150	96	90	86	218	48	72
168	324	412	286	166	100	62	74	219	60	86
171	376	424	318	167	68	54	62	220	82	108
172	390	438	362	181	108	98	94			
191	640	712	574	182	88	88	80			
193	764	820	640	183	72	64	68			

A review of the literature on this subject shows that different authors have reached conflicting conclusions about the effect of physical activity on blood cholesterol and phospholipids. Chailley-Bert, P. Labignette, and Fabre-Chevalier [6] observed a fall in blood cholesterol in persons who changed from a sedentary to an active life and who took up sport. E. S. Mnukhina [1], in careful experiments on rats, found that muscular exertion of short duration was associated with an increase in phospholipids and cholesterol in the muscles, but that muscular exertion over a long period of time resulted in a fall in blood phospholipids and cholesterol. She concluded that muscular activity involves consumption by the active muscles of lipids and of cholesterol. However, G. Amelotti [3] found in experiments on rats that rapid exhaustion leads to a fall in the blood lipids, and slow exhaustion to a rise.

Inasmuch as all of the authors just mentioned conducted careful research, we decided to study the blood cholesterol of our rabbits immediately after they had been exercised. We measured the blood cholesterol of 6 rabbits of group II and 6 rabbits of group III before exercise, immediately afterwards, and 3 hours afterwards (Table 1). We found that the blood cholesterol of the rabbits of group III (which had not been fed cholesterol) fell immediately after exercise.

The blood cholesterol of the rabbits of group II (which had been fed cholesterol) rose immediately after exercise, but three hours later fell to below its original level. We made these investigations during the fourth and fifth months of the experiment, when the rabbits had become used to running on the wheel; we can therefore exclude the operation of emotional factors which, as several investigators have shown [7-9], cause a temporary rise in blood cholesterol. That emotional factors did not play a role is further shown by the fact that the

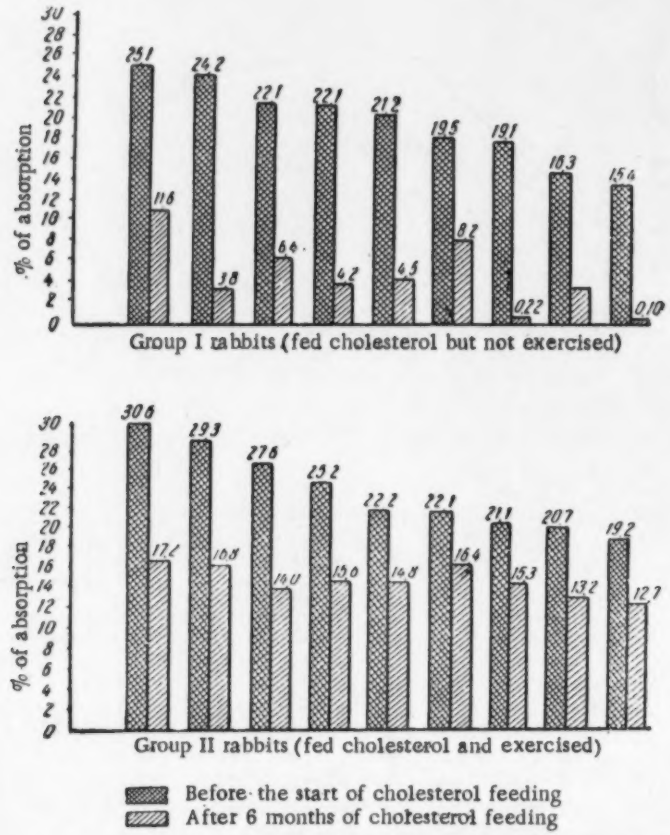

Fig. 2. Absorption of radioactive iodine (I^{131}) by the thyroid gland of rabbits of groups I and II.

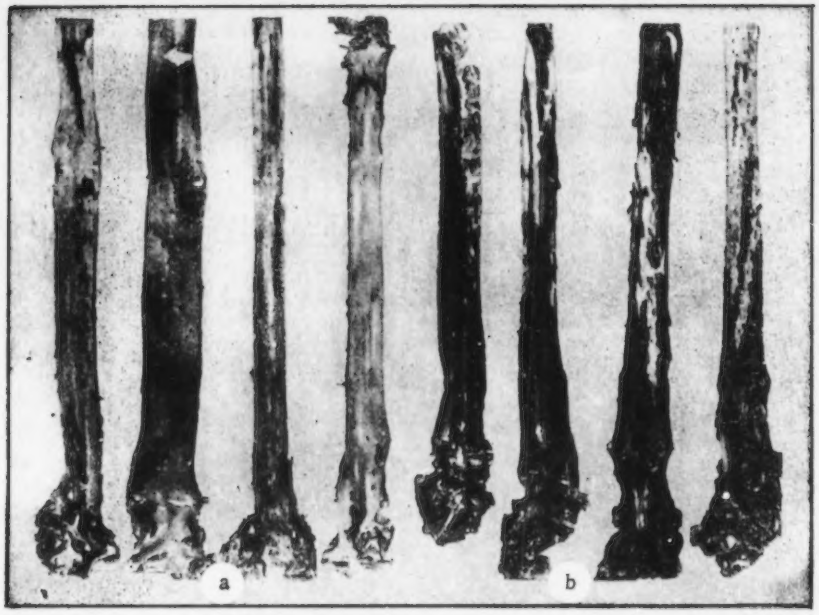

Fig. 3. Aortas of rabbits which had been fed cholesterol and made to exercise (a), and aortas of rabbits which had been fed cholesterol but not made to exercise (b).

blood cholesterol of the rabbits of group III fell immediately after exercise.

In addition we placed 3 healthy rabbits on the wheel and found a temporary rise in their blood cholesterol, which can only be explained by the operation of emotional factors (reaction to the noise of the motor, the motion of the wheel). In exact experiments in which animals are made to do exercise of a type to which they are unaccustomed, the emotional element must be taken into account.

Our findings, that rabbits fed cholesterol and made to exercise developed hypercholesterolemia more slowly and had lower levels of blood cholesterol, may be explained by an increased consumption of cholesterol by active muscles, with a consequent lowering of its level in the blood. Several authors are of this opinion [1, 4, 5].

In our study of thyroid functioning by means of I^{131} we found in the rabbits of group I (which had been fed cholesterol but not made to exercise) a great decrease in radioactive iodine absorption toward the end of the experiment, whereas the decrease in the case of the rabbits of group II (which were fed cholesterol and made to exercise) was unimportant (Fig. 2).

As is known, muscular activity increases the activity of the endocrine glands, particularly the thyroid gland, and causes an acceleration of the metabolic processes of the organism.

Atherosclerosis of the aorta was very marked in the rabbits of group I (which had been fed cholesterol but not made to exercise). Large confluent atheromatous plaques were prominent on the surface of the aorta all along its length and were particularly extensive in the arch and ascending branch. We evaluated the atherosclerosis of this group at +++ (Fig. 3).

We also found marked atherosclerosis of the coronary arteries, mainly in the intramyocardial branches. The atheromatous plaques greatly narrowed the lumen of the vessels. In the large subepicardial branches we more often found lipid infiltration of the intima, although very large atheromatous plaques did occur in some of the animals. We evaluated the atherosclerosis of the coronary arteries at ++.

In examining the aortas of the rabbits of group II (which had been fed cholesterol and made to exercise) we found remarkably little atherosclerosis in most cases. The occasional atheromatous plaques were situated

TABLE 2

Atherosclerosis of the Aorta and Coronary Arteries (Group I: control group, rabbits fed cholesterol; Group II: rabbits fed cholesterol and made to exercise)

Nos. of rabbits group I	Atherosclerosis		Nos. of rabbits group II	Atherosclerosis	
	aorta	coronary arteries		aorta	coronary arteries
120	+++	++	165	+	+
121	+++	++	168	+	+
122	+++	++	171	+	+
123	+++	++	172	+	+
125	+++	++	175	+	++
126	+++	++	177	++	++
127	+++	++	179	+	++
128	+++	++	186	+	+
129	++	+	188	+	+
130	+++	+	190	++	++
131	+++	++	191	++	++
132	++	+	102	+	+
			193	++	++
			194	++	++

mainly in the ascending branch of the aorta, in the arch, and at the orifices of the intercostal arteries. We evaluated most cases of atherosclerosis of the aorta in group II at +, the remainder at ++ (Table 2).

Atherosclerosis of the coronary arteries in group II affected mainly the intramyocardial branches of the left and, less often, the right coronary arteries. There were no atheromatous plaques in the large subepicardial branches.

We thus found the same range of atherosclerotic changes (+ and ++) in the coronary arteries of the rabbits of group I and group II.

However, the proportion of animals with only slight alterations of the coronary arteries (+) was much higher (7 out of 14) in the group which had been made to exercise (group II) than in the control group (group I), which had not been made to exercise (3 out of 12).

The relatively small difference in the atherosclerotic changes in the coronary arteries of the two groups of rabbits can be explained be the heavy functional load on the coronary circulation of the rabbits which were made to exercise. This explanation is in agreement with the findings of M. Schmidtmann [11] and E. Pfleiderer [10].

Our findings support the view that daily physical exercise hinders the development of hypercholesterolemia. This effect appears to be associated with increased activity of the thyroid gland, which occurs in connection with physical exertion. The physical exercise done by one group of cholesterol-fed rabbits resulted in these rabbits developing less atherosclerosis of the aorta than did a control group of cholesterol-fed rabbits which did not do exercise and which developed pronounced atherosclerosis of the aorta. The difference between the two groups in the development of atherosclerosis of the coronary arteries was less significant, probably because of the heavy functional load on the coronary circulation of the rabbits which were made to exercise.

SUMMARY

The effect of physical strain on the development of atherosclerosis of the aorta and of the coronary arteries was studied in rabbits.

It was established that physical exercises (daily running of rabbits) combined with cholesterol feeding

inhibits the development of hypercholesterolemia and, therefore, the development of aortic atherosclerosis is not very intense. The activity of the thyroid gland is increased under the effect of physical strain. This possibly has an effect on the condition of lipid metabolism.

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CHANGES IN THE VISCO-ELASTIC PROPERTIES OF MUSCLES IN CONTRACTURES AFTER IMMOBILIZATION

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While engaged in the study of the pathogenesis of contractures arising as a result of immobilization of the limbs, several workers have been able to discover a reflex mechanism in the first phase of their development. The problem of the mechanism of their maintenance in later stages is not so clear.

In view of the hypothesis that as a result of prolonged immobilization, changes take place also at the periphery, including in the muscles themselves, we undertook an investigation of the visco-elastic properties of muscles during immobilization, these properties being indirect indexes of the state of the colloid structures of the muscles.

EXPERIMENTAL METHOD

Experiments were performed on isolated muscles of the rabbit, in which one of the hind limbs (the left) had been immobilized for periods of 1 to 5 months as a preliminary measure. Immobilization was accomplished by the application of a plaster cast, fixing the three joints of the limb in the position of either maximum flexion or maximum extension. The muscles of the limb which was not immobilized served as controls. The tibialis anterior and plantaris muscles of the leg were examined. Muscle preparations were carried out on animals in a state of deep anesthesia, in order to remove the influence of strong reflex stimulation on the initial condition of the muscles. The isolated muscles were placed in Ringer-Locke solution, warmed to a temperature of 36-40°C and aerated uniformly with oxygen or, less commonly, with atmospheric air. Both muscles for comparison were placed in the solution at the same time, so that strictly identical experimental conditions were observed.

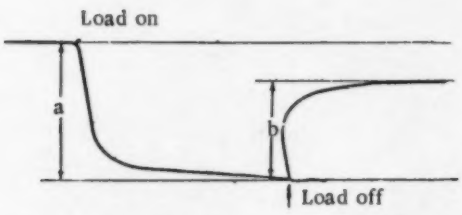

Fig. 1. Scheme of analysis of the curve of stretching.
a) Magnitude of maximum lengthening (degree of stretching); b) degree of restoration of length one minute after removal of the load.

In order to assess the visco-elastic properties, we recorded on a kymograph the curve of change in length of the muscle after the sudden application of a load and after its equally sudden removal. For this purpose the muscle, with its proximal end fixed to a glass rod, was immersed in a glass containing the solution, and its tendon connected to one arm of a myograph; the load was suspended from its other arm. For stretching the plantar muscles the weight applied was usually 25 g, and for the anterior tibial muscles – about 15 g. Each muscle was stretched for one minute, after which the load was removed. The curve of restoration of its length was also recorded for one minute. The curves obtained were analyzed in respect to the following indexes (Fig. 1): 1) the magnitude of the maximum lengthening (Fig. 1,a) as a

Mean Values of the Degree of Stretching and Recovery of the Muscles of the Leg in Normal Rabbits and in Rabbits After Fixation of the Left Hind Limb (in %)

Serial No.	Experimental conditions	Degree of stretching of the muscles				Degree of recovery of the muscles			
		Tibialis anterior		Plantaris		Tibialis anterior		Plantaris	
		left	right	left	right	left	right	left	right
1	Without immobilization (normal rabbits)	20.7	20.5	7.1	7.3	81	81	69	70
2	Immobilization of the left hind limb in a position of flexion	11.4	19.0	4.3	6.2	72	71	70	69
3	Immobilization of the left hind limb in a position of extension	10.7	21.1	4.6	8.2	65	66	61	60

percentage of the initial length of the muscle (degree of stretching) and 2) the degree of restoration of its length one minute after removal of the load (Fig. 1, b) as a percentage of the level of stretching (ratio b:a).

The experimental results were treated by the method of variational statistics. The degree of significance of the variations in the experimental results from the controls was calculated from the tables of Fisher and Student.

EXPERIMENTAL RESULTS

In the first series of experiments to determine the limit of variation of the visco-elastic properties of fellow muscles of the right and left limbs, the curves of stretching of muscles isolated from the limbs of healthy animals were compared. Altogether 15 animals were examined in this series. The experimental results showed that the variations in the level of stretching in symmetrical muscles did not on the average exceed tenths of one percent, although in isolated cases variations of 2-3% were observed in the tibial muscles and of 1-2% in the plantar muscles. Average values for stretching of muscles in the control series of rabbits are shown in the table.

It can be seen from the table that the anterior tibial muscles are approximately 3 times as elastic as the plantar muscles, which is in agreement with their morphological characters: the anterior tibial muscle has long fibers, arranged in parallel, whereas the plantar muscle has a pinnate structure and, consequently, shorter fibers and a larger physiological section.

In the second and third series of experiments the visco-elasticity of the muscles was investigated in limbs subjected to immobilization. In evaluating the results of these series of experiments it was essential to take into consideration that as a result of immobilization there takes place at times a considerable diminution in the mass of muscles, as expressed by loss of weight and reduction in volume. On the basis of reports in the literature [2, 5] and also of the results of our own observations, it must be mentioned that the diminution in mass of functionally different muscles is expressed to a different degree, and depends on the length of immobilization and the position in which the limb was fixed. Thus in this particular series of experiments we found a greater diminution in the muscles, and at earlier periods, when the limbs were fixed in the position of extension, and always to a greater degree in the plantar muscles. It follows from the above that on stretching symmetrical muscles of the control and experimental limbs with equal loads per unit cross section, different forces acted on each of them. For this reason the level of stretching reflected not only the visco-elastic properties of the muscle but also the size of the load acting per unit cross section.

To be able to compare the results it was necessary to calculate the magnitude of stretching of the control and experimental muscles, taking into consideration the cross section of the muscle. The area of cross section was calculated from photograms of the muscles, in natural size, in which the width of the muscles was measured. In view of the hypothesis that diminution of volume takes place uniformly in all directions, the thickness of the muscle can be calculated from its width. The average ratio of width to thickness was determined on the basis of measurements in 10 control experiments. The shape of the cross section was taken conventionally to be an

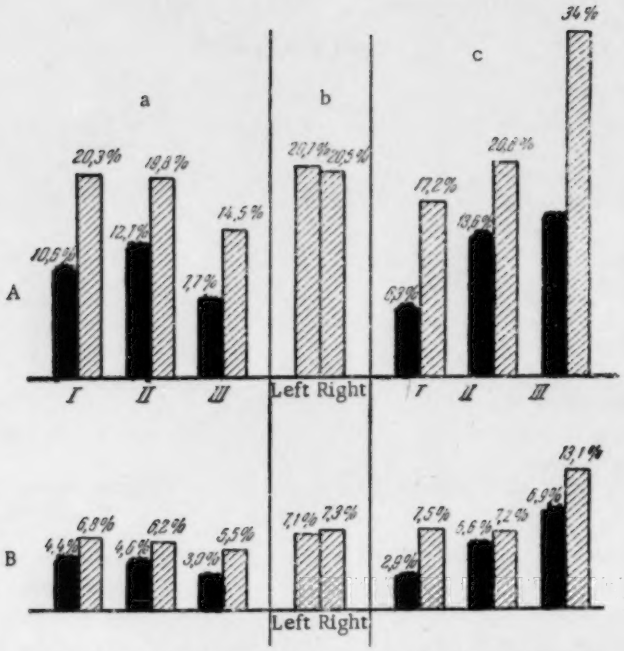

Fig. 2. Changes in the stretching of muscles after different periods of immobilization.
A -Anterior tibial muscles: a) in a position of flexion; b) average degree of stretching of muscles in normal rabbits; c) in a position of extension. Periods of fixation: I – 2 months; II – 3-4 months; III – $4\frac{1}{2}$ -5 months. B - plantar muscles: a, b, c) as in A. Periods of fixation: I – 1 month; II – 2 months; III – $4\frac{1}{2}$ months.

ellipse, and the area calculated by the well-known formula. The size of the load per 1 mm^2 section, and the degree of stretching, which is proportional to this, were subsequently calculated.

In the second series of experiments observations were made on animals whose limbs had been fixed in a position of flexion. The series consisted of 24 experiments on rabbits with varying periods of fixation: $4\frac{1}{2}$ -5 months – 4 rabbits, 3-4 months – 16 rabbits, and 2 months – 4 rabbits.

The table shows the average values of stretching for the muscles of the control and experimental limbs.

As seen from the table, the degree of stretching of the muscles of the unimmobilized limb almost coincides with the results of the control experiments. The stretching of the muscles of the immobilized limb was reduced by comparison with the muscles of the control limb by 1.7 times for the tibialis anterior muscle and by 1.2 times for the plantaris muscle. The difference in stretching of the plantar muscles was less significant. If the degree of stretching is examined without consideration of the changing cross section of the muscle in the immobilized limb, then this difference is reduced for the anterior tibial muscles: the muscle of the immobilized limb has an average extensibility of 14.9% (in place of 11.4%) with an extensibility of the muscles of the control limb of 19.0%. For the plantar muscles, if the extensibility is compared without taking into consideration the changing cross section of the muscle, this difference does not in general take place: the muscle of the control limb is stretched on the average by 6.2%, and the muscle of the immobilized limb by 6.3% (in place of 4.3%). This may be explained by the fact that, because of the pinnate structure of the plantaris muscle, the alteration in its physiological cross section is far greater than could have been estimated by the method of calculation described.

If the changes in the visco-elastic properties are examined in relation to the periods of fixation of the limbs, the relationships shown (in Fig. 2, a) are observed. As the duration of immobilization increases, the

elasticity of the muscles is diminished not only in the immobilized limb but also to some extent in the control limb which is not immobilized; for this reason the difference between the elasticity of compared symmetrical limbs does not change so sharply or even remains constant as the period of immobilization is increased. This is characteristic of both the plantar and anterior tibial muscles.

In the last, third, series of 9 experiments, the visco-elastic properties of muscles were investigated after fixation of the left limb in a position of extension. The periods of fixation were as follows: 1 rabbit for 4½ months, 5 rabbits for 2 months and 3 rabbits for 1 month.

It must be pointed out that after fixation of the limb in this position, a contracture develops after only one month, i.e. sooner than after fixation in a position of flexion, when signs of a persistent contracture appear only after 2-2½ months. The animals tolerated prolonged immobilization in a position of extension with difficulty, and often died. Thus after a period of fixation of 4½ months it was possible to keep only one rabbit out of five. The summarized results of this series of experiments are shown in the table (in series 3).

As seen from the table, the muscles of the control limb were stretched on the average to almost the same extent as the corresponding muscles of normal animals. The difference in elasticity between the control and experimental animals is greater here than in the case of fixation in the position of flexion. The elasticity of the anterior tibial muscles of the immobilized limb is reduced roughly to half, and that of the plantar muscles 1.8 times.

In the study of the character of the changes in the visco-elastic properties in relation to the period of fixation, the following regular feature is observed: the muscles of both the control and the experimental limbs show increased elasticity as their period of fixation is lengthened (Fig. 2, c). Thus in the muscles of the immobilized limb the least degree of stretching is observed after a period of fixation of one month (in the tibialis anterior muscle – 6.3%, in the plantaris – 2.9%), and the greatest – after a period of fixation of 4½ months (in the tibialis anterior muscle – 15%, in the plantaris – 6.9%). In the muscles of the control limb, after prolonged fixation (for 4½ months) the elasticity is increased by more than 1½ times in comparison with normal (i.e. the mean level of stretching in the control experiments).

Attention must be directed to the fact that fixation of one limb is not without an effect on the state of the muscles of the other limb. Examples of this fact have been observed in the literature [3].

In comparing the results of the second and third series of experiments (Fig. 2, a and c) it can be seen that the changes in the elasticity with increasing periods of fixation in flexion and extension are opposite in nature: after fixation in flexion the elasticity falls, but after fixation in extension it rises, although it remains for long periods below normal. As the elasticity of the muscles of the immobilized limb changes, so also correspondingly does the elasticity of the muscles of the control limb. The difference in elasticity between the muscles of the control and the immobilized limbs is greater in the case of fixation in the position of extension.

Comparison of the degree of restoration of the initial length of the muscles of the control and immobilized limbs (see table) shows that in all cases it is almost the same in the symmetrical muscles. Consequently the relationship between the "viscous" and the elastic properties is the same in the muscles compared. Comparison of the degree of recovery of the muscles in the fixation experiments (Fig. 1, a and c) with the results of experiments on normal muscles (Fig. 1, b) confirms that as a result of immobilization the degree of recovery falls, and again to a greater degree in experiments in which fixation is carried out in the position of extension.

Thus a comparison of the degree of the changes caused by immobilization in positions of flexion and extension demonstrates that fixation in the position of flexion is accompanied by smaller disturbances in the muscles of the immobilized limb than is fixation in the position of extension, and after longer periods of fixation it has a smaller effect on the visco-elastic properties of the muscles of the control limb.

The explanation of this fact may be that flexion is more natural for the rabbit.

A state of full extension is practically never observed. Presumably when a limb is put into an unnatural position, disturbances of the properties of its muscles develop more rapidly and to a greater degree.

SUMMARY

The author compares the degree of extension of the isolated symmetrical muscles of rabbits, one of the

extremities of which was subjected to immobilization in the position of flexion or extension for the period of 1-5 months. Immobilization causes the decrease in the stretching capacity in muscles of the fixated extremity, as compared to control. This difference was greater in cases of fixation in a position of extension. In prolonged periods of immobilization the changes were noted in the stretching extension capacity of the control extremity, i. e. an increase in the stretching capacity in fixation in the position of extension and its decrease in the position of flexion. The degree of these changes was also found to be greater in fixation in the position of extension, i. e. in the position less "physiological" for rabbits.

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THE MECHANISM OF THE DISORDERS OF RESPIRATION IN TOXIC FORMS OF DIPHTHERIA

COMMUNICATION I. THE EFFECT OF DIPHTHERIA TOXIN ON THE FUNCTIONAL STATE OF THE RESPIRATORY CENTER IN GUINEA PIGS

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As clinical observations show [7, 8, 12], in toxic forms of diphtheria respiratory disorders play a leading part. Paralyses of the respiratory muscles – the intercostals and diaphragm [6] – are dangerous to life and are often complicated by pneumonia. In laryngeal diphtheria there is often spasm of the muscles of the larynx. The mechanism of spasm is connected with the increased excitation of the peripheral receptors on the one hand, and on the other with factors of a conditioned reflex and psychogenic order [3, 12, 14].

Very few experimental investigations have been devoted to the study of the respiratory disorders in diphtheria. N. M. Porubinovskaia [9, 10] put forward the suggestion of inoculation of rabbits with a diphtheria culture through a puncture of the larynx as an experimental model of laryngeal diphtheria. Her morphological research supports the opinion of clinicians that the cause of stenosis and asphyxia in laryngeal diphtheria is a neuroreflex spasm of the muscles of the larynx.

Enriquez and Hallion [16] point out that disturbances of respiration under the influence of diphtheria toxin are associated with a fall in the blood pressure, which is the direct cause of death of the animals.

V. O. Krasnov [5] emphasizes the importance of disorders of respiration in the picture of experimental toxic diphtheria in cats and dogs. The author associates a lesion of the inspiratory center with paralysis of the endings of the phrenic nerve. At the same time, this worker points out that the excitation of the respiratory center gradually and progressively falls in toxic conditions, and death of the animals in experimental diphtheria occurs from respiratory paralysis.

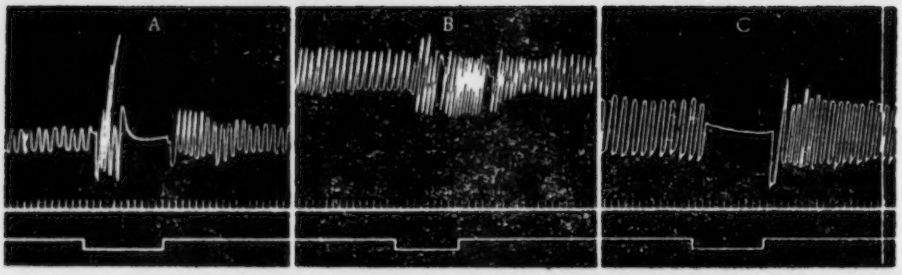
Recently V. N. Abrosimov [1, 2] has studied the changes in the respiratory movements during toxic diphtheria in rabbits and cats. He used an induction current to stimulate the central part of the vagus and also the sciatic nerves, and found that in cases of poisoning with diphtheria toxin the threshold of stimulation rises; the author associates this with reduced excitation of the respiratory center.

The aim of the present investigation is to study the excitation and the physiological lability of the respiratory center during the action of diphtheria toxin on guinea pigs.

Since an important role in the reflex regulation of respiration is played by the vagus nerve, we employed stimulation of its central segment to judge the functional state of the respiratory center.

EXPERIMENTAL METHOD

Altogether 39 experiments were carried out on 26 guinea pigs. The experiments were performed without anesthesia, or under light urethane anesthesia. The respiration was recorded by a Marey's capsule connected to



Changes in respiration in guinea pigs under the influence of diphtheria toxin.
A – Effect of stimuli at a frequency of 80 cps before injection of toxin; B – the same 27 minutes after injection of 0.5 MLD of diphtheria toxin; C – the same 19 minutes after a second injection of toxin (20 MLD). Significance of the curves (from above downward): respiration (inspiration – downwards), time marker (1 second), stimulus marker.

a cannula inserted into the trachea. The right vagus nerve was divided and its central end stimulated by means of an electric current. In some experiments both vagus nerves were divided. The source of stimulation was a tube generator of rectangular stimuli in which the current voltage could be changed between limits of 0 and 60 v, and the frequency of the impulses from 1 to 1400 cps. The state of excitation of the respiratory center was judged by determination of the threshold of stimulation of the afferent fibers of the vagus nerve at a frequency of 10 cps before obtaining minimal changes in respiration. Subsequent stimulation was carried out at a current voltage three times above the threshold level. The physiological lability of the respiratory center was judged by changes in the character of respiration in response to stimulation of the central end of the vagus nerve by current impulses of varying frequency. Diphtheria toxin was injected intraperitoneally, as a rule in a dose of 1 MLD, but in individual experiments in doses of 0.1 to 100 MLD. These indexes of the physiological activity of the respiratory center were determined repeatedly before the injection of toxin and at various intervals of time after its injection.

EXPERIMENTAL RESULTS

The threshold of stimulation of the central end of the vagus nerve before the first minimal changes in respiration (usually a quickening and deepening) varied insignificantly, and was on the average 0.9 v in the experiments without anesthesia and 1 v in the experiments on animals feebly anesthetized with urethane.

The use of different frequencies of stimulation resulted in the discovery of the following general relationship. At low frequencies (10-20 cps) the respiration of the guinea pigs was quickened and deepened, and with increase of the frequency (40-60 cps) a biphasic reaction was usually observed: the rate of respiration increased and then began to fall. On increasing the frequency of stimulation to 60-80 cps, the respiration rate fell. Finally, at even higher frequencies (80, or even 100-120 cps) respiration ceased. Cessation of respiration developed as a rule at the beginning or in the middle of the phase of inspiration, and lasted throughout the whole period of stimulation (usually 10 seconds). Sometimes at high frequencies (140 or 160 cps) respiration was restored very soon after the end of the period of stimulation.

During the action of diphtheria toxin the threshold of stimulation of the central end of the vagus nerve is usually raised. For example, in one of the experiments the initial threshold of stimulation at a frequency of 10 cps was 1 v, and 30 minutes after the intraperitoneal injection of only 0.1 MLD of toxin it was 2.25 v. If the diphtheria toxin (1 MLD subcutaneously) was injected earlier, many hours before the experiment, then for the first 24 hours after injection the threshold of stimulation of the central end of the vagus nerve corresponded roughly to the threshold in normal guinea pigs, or it was slightly above the normal value. At the same time, at the end of the second day after injection of toxin the threshold was sharply increased, sometimes reaching 9 v. This demonstrates a fall in the excitation of the respiratory center during the action of diphtheria toxin.

The use of different frequencies of stimulation showed that after the action of toxin the limit of appearance of biphasic reactions, slowing of the rhythm and cessation of respiration is displaced towards the higher frequencies (see Figure, A, B). In toxic conditions, during the action of high frequencies of stimulation it is

The Influence of Diphtheria Toxin on the Physiological Lability of the Respiratory Center

Changes in respiration	Frequency (in cps)	
	before action of toxin	after action of toxin
Transition from quickening to slowing	40-60	80-140
Slowing	60-80	100-160
Cessation	80-120	120-180

easier to obtain quickening than slowing of the respiratory rhythm.

The changes in the physiological condition of the respiratory center in this direction are quite regular and are duplicated in all the experiments without exception. At frequencies which before injection of toxin caused a slowing of the respiratory rhythm, after injection a quickening and strengthening of respiration was observed. The figures given in the right hand column of the table show the changes in respiration observed from 15-30 minutes to 1 hour and more after the intraperitoneal injection of diphtheria toxin.

After the action of large doses of diphtheria toxin – from 4 to 20 and 100 MLD, and also in some cases from 1½ to 2 hours and more after the ordinary dose used (1 MLD)–another type of reaction could be observed. A shift in the optimal conditions for activation of respiration and for inhibition respectively took place in the direction of lower frequencies (see Figure, C).

The vagus nerve is known to play an essential part in the regulation of breathing of the animal (Hering-Breuer reflex). In order to judge the functional state of the respiratory center many research workers [4, 11, 13, 15, 17] have employed stimulation of the central end of this nerve. The investigations have been carried out mainly on rabbits, cats and dogs. We succeeded in observing the above-mentioned characteristic changes in respiration in guinea pigs by the action of stimuli of different frequencies, which are distinguished from the changes in respiration under similar conditions in other species of animals.

The fall in excitation of the respiratory center which we observed in guinea pigs under the influence of diphtheria toxin is in agreement with the reports in the literature [1, 2, 5] on the fall in the excitation of the respiratory center of rabbits and cats under the same conditions. Our experiments carried out on rabbits also confirm these findings.

Displacement of the zone of optimum frequency in the direction of the more powerful and frequent stimuli is regarded by physiologists, studying the physiological lability of the cerebrospinal centers, as an index of increase in the physiological lability of the nerve centers. By analogy with this view it may be postulated that in our experiments the first phase of the changes in the functional condition of the respiratory center under the influence of diphtheria toxin is characterized by an increase in physiological lability. In the second stage, by the action of large doses of toxin, a change takes place in the optimum for stimulation and subsequent inhibition of respiration in the direction of the lower frequencies, and this evidently is an indication of a reduction in the physiological lability of the respiratory center.

The biphasic changes in physiological lability of the respiratory center which we observed after the injection of diphtheria toxin are peculiar to the action of diphtheria toxin, since in control experiments with Martin's broth and typhoid toxin results close to those described above were not obtained.

SUMMARY

The effect of diphtheria toxin on the functional condition of the respiratory center was studied in guinea pigs. It was demonstrated that diphtheritic intoxication is associated with decreased excitability of the respiratory center and with vast changes in its physiological lability. The first phase of intoxication is characterized by a constant increase in the physiological lability of the respiratory center, while the second – by its decrease.

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ADRENALIN-LIKE SUBSTANCES IN MYASTHENIA

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The neuro-humoral theory of nerve excitation is largely responsible for the discovery of the pathogenesis of myasthenia. The elucidation of the role of acetylcholine in the process of excitation has led to a greater understanding of the intimate mechanisms of the motor disorders in myasthenia. On this basis considerable success has been achieved in the development of pathogenetically justified treatment, directed at regulation of the disturbed acetylcholine metabolism by means of the use of the so-called anticholinesterase preparations.

Numerous investigations on this problem have been devoted mainly to an exploration of the various possible causes of the acetylcholine deficiency: to its destruction, its inadequate synthesis, and to the blockade by curare-like substances, i. e. to factors leading eventually to a state of so-called "functional asynapsia" [3]. Investigations of this sort are characterized by some degree of one-sidedness. For a comprehensive investigation of the problem it is necessary to account for the role of another hormonal factor which is of essential importance in the normal processes of neuromuscular excitation, namely the role of adrenalin and of adrenalin-like substances. An explanation of the role of these factors in the mechanism of the disorders and of the restoration of function in myasthenia is all the more necessary since the activity of the cholinergic and adrenergic systems in the processes of neuromuscular activity in a number of cases is not antagonistic but synergistic.

In connection with these problems, the aim of our investigation was to study the changes in adrenalin and adrenalin-like substances in myasthenia in association with the use of drugs causing clinical improvement of myasthenic patients. Such factors include anticholinesterase preparations (proserin – prostigmin methylsulfate) and the adrenocorticotropic hormone (ACTH), and x-ray irradiation of the thymus gland.

EXPERIMENTAL METHOD

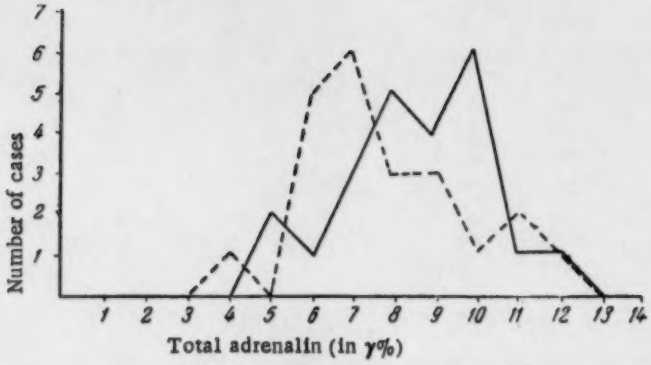
The adrenalin and adrenalin-like substances of the venous blood were determined by the method of Shaw [9] as modified by Utevskii and Butom [6]. The final staining was estimated colorimetrically on a type SF 4 spectrophotometer at a wavelength of 630 mμ. In this way the following fractions of adrenalin-like

TABLE 1

Adrenalin-Like Substances in the Blood of Normal Persons and of Patients with Myasthenia

Name of group	No. of cases	RAS		TA		CSP	
		M	m	M	m	M	m
Normal	24	7,8	0,40	8,0	0,39	0,9	0,06
Myasthenia	23	6,7	0,33	7,4	0,42	0,9	0,08

Symbols: M – arithmetic mean; m – mean square error of the arithmetic mean.



Total adrenalin content in the normal subject and in myasthenia;
——— normal; - - - - - myasthenia.

substances were determined: 1) in acid medium – the reduced adrenalin-like substances (RAS); 2) the content of dehydroadrenalin-like substances, by the difference between the content of RAS in the presence and absence of ascorbic acid; the total content of both fractions is referred to as "total adrenalin" (TA). The ratio between the values obtained by treatment with alkali and acid is an expression of the "coefficient of specificity" (CSP), which enables the relative content of adrenalin and of as yet unidentified adrenalin-like substances (chromogens) in the RAS fraction, a component of which is noradrenalin, to be judged. When the CSP > 2 it is considered that the RAS contains only adrenalin, and when the CSP < 1 adrenalin is absent. When 2 > CSP > 1 it is considered that the RAS is a mixture of adrenalin and of as yet unidentified adrenalin-like substances. In this paper the values of the RAS, TA and CSP are given in tables. Observations were made on 24 apparently healthy persons and on 23 patients with myasthenia before the commencement of treatment, in a state of myasthenic exhaustion.

EXPERIMENTAL RESULTS

The results of statistical analysis of the experimental findings are shown in Table 1.

Analysis of the figures in Table 1 shows that in myasthenia there is a tendency for the RAS content of the blood to fall. Under these circumstances the value of the CSP shows that both in the normal subject and in patients with myasthenia free adrenalin does not appear in the blood and only chromogens may be detected (CSP < 1). The tendency for the adrenalin-like substances to fall in myasthenia is also shown by the Gaussian distribution curves of the total adrenalin content in the normal subject and in myasthenia (see Figure). Some reduction in the content of adrenalin-like substances in the blood of patients with myasthenia we regard as the result of the acetylcholine deficiency in myasthenia.

The study of the movements of the content of adrenalin-like substances in the blood of 21 patients (35 observations) before and 45 minutes after the subcutaneous injection of 2 ml of a 0.05% solution of proserin (when a sudden improvement in the clinical condition of the patients took place) showed that in the majority of cases (29 out of 35) there was an increase in the total content of adrenalin-like substances, mainly due to the unoxidized fractions. Under these conditions the CSP of these simple changes was not ascertained. In different patients the increase in the adrenalin-like substances differed (from 0.3 to 4 γ%) and varied in the same patient during repeated examinations, but as a rule there was a clear tendency towards an increase in the adrenalin-like substances.

In Table 2 are shown the results of a study of the effect of proserin on the content of adrenalin-like substances in the blood – the arithmetic mean values of the RAS, TA and CSP, their mean square errors and coefficients of significance.

It can be seen from Table 2 that after the injection of proserin into the patient there is observed an increase in the RAS and TA, and the differences observed are statistically significant, $t \geq 3$.

We also observed similar changes in the content of adrenalin-like substances in the CSF in three patients under observation 10 minutes after the subcutaneous injection of proserin (Table 3).

TABLE 2

Effect of the Injection of Proserin on the Content of Adrenalin-Like Substances in the Blood of Patients with Myasthenia*

Fractions tested	No. of observations	Conditions	M	m	t
Reduced adrenalin-like substances	35	Before injection of proserin	6.3	0.36	
	33	After injection	8.6	0.51	3.7
Total adrenalin	35	Before injection of proserin	8.1	0.59	
		After injection	10.2	0.43	3.0
Coefficient of specificity		Before injection of proserin	1.1	0.10	
		After injection	1.0	0.04	0.9

Note: M – arithmetic mean; m – mean square error of the arithmetic mean; t – coefficient of significance calculated from the formula:

$$t = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}}.$$

when $t \geq 3$ the material is considered to be significant.

* The study of the effects of proserin was carried out in patients, both undergoing treatment and not.

TABLE 3

Adrenalin-Like Substances in the Blood and Cerebrospinal Fluid of Patients with Myasthenia Under Treatment with Proserin, ACTH and X-rays

Surname	Material examined	Treatment	RAS, γ %		TA, γ %		CSP	
			before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
P - in	Cerebrospinal fluid	Injection of proserin	4,3	6,3	—	—	—	—
K - ov	The same	The same	3,5	4,0	—	—	—	—
N - ov	" "	" "	6,7	7,4	—	—	—	—
N - ov	Blood	X-ray therapy	7,0	13,4	7,0	17,0	1,1	0,9
Sh-va	"	The same	2,5	7,1	2,5	7,8	1,2	0,8
M-in	"	Treatment with ACTH	6,8	16,1	6,8	18,2	1,0	1,1
M-in	"	The same	10,6	3,8	10,6	5,6	1,4	1,7
K - na	"	" "	10,0	14,0	11,7	14,0	0,8	0,9
B - va	"	" "	4,8	8,5	5,7	9,9	0,7	0,8
M-in	"	One injection of ACTH	10,6	9,4	10,6	12,7	1,4	1,1
Sh-ev	"	The same	12,2	13,0	12,2	13,0	0,4	0,7

The injection of ACTH (both as a single injection of 20 units and after a course of 6 daily injections of 40-60 units) led to an obvious increase in the content of adrenalin-like substances in the blood (see Table 3).

A course of irradiation of the thymus gland with x-rays leads to clinical improvement and is accompanied in these patients by an increase in the content of adrenalin-like substances in the blood too.

The results given are not equivalent in a quantitative respect for the different forms of treatment.

Nevertheless the identity of the humoral changes, leading in the same direction after different forms of treatment, and the similarity of the clinical effects suggest that the increase in the content of adrenalin-like substances is an important functional factor in the improvement of the condition of patients suffering from myasthenia.

It must be pointed out that the factors under study – proserin, ACTH and x-ray therapy – affect in different ways the increase in the reserves of acetylcholine (proserin suppresses cholinesterase activity, ACTH increases acetylcholine synthesis [10], x-ray therapy suppresses the formation in the thymus of a factor inhibiting acetylcholine activity [11]).

An increase in the acetylcholine in the preganglionic formations of the sympathetic nervous system and the parasympathetic endings innervating the medullary layer of the adrenals, leads to an increase in the reduced forms of adrenalin-like substances. Accumulation of adrenalin-like substances may have a stimulating action on the oxidative and glycolytic processes in muscle and on its working capacity [1, 2, 5, 8].

The results obtained give grounds for postulating that the action of proserin, ACTH and x-ray therapy, while improving the condition of a myasthenic patient, may also produce an increase in the content of adrenalin-like substances, leading to improvements in muscle metabolism.

It has to be pointed out that the investigations of L. B. Perel'man, N. V. Raeva and R. A. Stavitskaia [4] on a biological test object demonstrated the regulatory role of anticholinergic drugs on sympathetic as well as acetylcholine effects.

Our investigation has confirmed this and has also enabled us to understand the positive action of ephedrine in combination with proserin in overcoming myasthenic disorders. Ephedrine is known to inhibit the destruction of adrenalin by aminooxidase, thereby bringing about its accumulation [7].

Our findings stress the importance of combined investigation of both mediators in the study of the pathogenesis and the mechanism of restoration of function in myasthenia.

SUMMARY

The majority of the studies on the significance of neurohumoral factors in the pathogenesis of myasthenia were limited to the ascertaining of the role played by acetylcholine. This work was devoted to the other mediator factor – adrenalin and adrenalin-like substances. An association was demonstrated between the shifts of the adrenalin-like substances and the changes in the clinical conditions of the myasthenic patients. An increase in the concentration of adrenalin-like substances was noted in the blood after treatment by proserin, ACTH and X-ray therapy.

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BIOCHEMISTRY AND BIOPHYSICS

CONVERSION OF β-ALANINE IN VIVO AND IN ISOLATED LIVER TISSUE

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Until recently the paths of conversion of β-alanine in the animal body have been uncertain in many respects.

In previous reports [1, 2] it was shown that the following mechanism of decomposition of β-alanine is present in the kidney tissue of rats:

β-alanine
↓ pyruvic acid
α-alanine
↓ α-ketoglutaric acid
glutamic acid
↓
NH_3

In the present paper we give the results of experiments studying conversion of β-alanine in liver tissue of rats and also in vivo.

EXPERIMENTAL METHOD

Experiments with rat liver. The experiments were carried out with rat liver pulp (rats weighing 120-140 g). A sample of the pulp weighing 600 mg was placed in 3.4 ml of a phosphate-saline mixture free from calcium ions [4]. The total volume of the mixture of reagents was 4 ml and its pH 7.1. The experimental sample contained 40 μM of β-alanine. Incubation was carried out in an atmosphere of oxygen at 37°C for 160 minutes. Proteins were precipitated by heating the samples in a boiling water bath for 2 minutes, after which the reagent mixture was rapidly cooled in ice water and centrifuged. In the protein-free centrifugate thus obtained, the urea and ammonia were estimated. These estimations were made by Conway's method. The urea was estimated by the quantity of ammonia given off as a result of the action of urease. The amino acids were estimated by the paper chromatography method [7, 9].

Experiments on the intact animal. Experiments were performed on four young rats with marked vitamin B_6 deficiency [1] and on four healthy rats. The rats were specially selected to be of the same weight – 170 g each. Throughout the whole period of investigation both control and experimental rats were kept on a synthetic diet not containing vitamin B_6 [2]. Each experimental rat was given a daily intraperitoneal injection of 10 mg of isonicotinylhydrazide (INH) in physiological saline. Instead of INH, the control rats were injected with 100 μg of vitamin B_6. β-alanine was injected after determined intervals, also intraperitoneally and in a dose of 1 mg/g body weight of the animal. After receiving the injections of β-alanine, the rats were placed in a separate metabolic cage. The urine was collected according to the method of Levine and Smith [12] with the sole exception that in our experiments no liquid paraffin was poured into the urine collecting funnel, and toluene was added to the receiver in place of NaF. The 48-hour sample of urine and the distilled water used for thorough

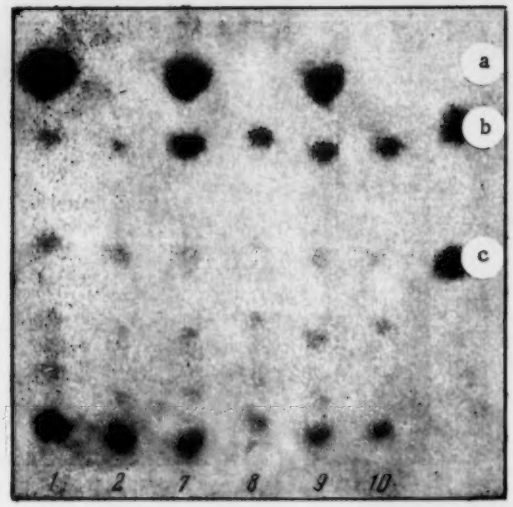

Fig. 1. Disappearance of β-alanine and excess formation of α-alanine during incubation of rat liver pulp in an atmosphere of oxygen.
1, 2 – Before incubation; 7, 8, 9, 10 – 160 minutes of incubation; a – β-alanine; b – α-alanine; c – glutamic acid.

washing of the cage and the funnel were combined, and the mixture was filtered through a Buchner funnel, evaporated down on a water bath and made up quantitatively to 50 ml in a measuring flask. The β-alanine in the solution was determined quantitatively by the chromatographic method [7, 9].

EXPERIMENTAL RESULTS

Experiments with rat liver tissue. During incubation of liver pulp from normal rats for 160 minutes in an atmosphere of oxygen, no excess ammonia could be determined in consequence of the added β-alanine. The chromatogram showed partial disappearance of β-alanine and some increase in α-alanine (Fig. 1), and it was clear that the quantity of β-alanine lost was greater than the α-alanine formed. It could be postulated that β-alanine is utilized in the liver tissue for the synthesis of urea [8, 11].

The experiments which were carried out confirmed this hypothesis. As may be seen from Table 1 the quantity of surplus urea formed was almost equivalent to the quantity of β-alanine disappearing, and furthermore, as has already been mentioned, a slight surplus accumulation of α-alanine also took place.

Roberts and Bregeff [14] reported that in preparations of the liver and brain of mice a process of transamination of β-alanine and α-ketoglutaric acid takes place. It must be pointed out, however, that these workers used unpurified enzyme preparations in their work, and these may have contained proteinases, peptidases and transaminases. It is therefore impossible under these circumstances to exclude the possibility of the formation of different α-ketoacids under the influence of these enzymes from taking part in the process of transamination. For this reason the results described by these workers cannot, it seems to us, be regarded as sufficient proof that α-ketoglutaric acid is a primary acceptor of the amino-group of β-alanine.

Nor was the mechanism of decomposition of β-alanine in rat liver explained in our work. It is possible that in the liver of rats, by analogy with the renal tissue, pyruvate serves as the primary acceptor of the amino-group of β-alanine, and the α-alanine formed, directly or after transamination with ketoglutaric or oxalacetic acid, serves as a source of nitrogen used in the synthesis of urea [7]. This problem requires further study.

Experiments on the intact animal. It has previously been shown [7] that in kidney tissue of rats in vitro β-alanine takes part in a process of transamination with pyruvate. The findings described above suggest the possible conversion of β-alanine by transamination in liver tissue also. If in the intact animal in vivo, conversion of β-alanine is brought about by transamination, then in severe vitamin B_6 deficiency the elimination of β-alanine from the body in the urine in consequence of the pyridoxin deficiency must be greater than in control animals. After injection of pyridoxin into experimental rats their power of converting β-alanine must be restored.

TABLE 1

Conversion of β-alanine During Incubation of Liver Pulp of Healthy Rats (calculated per 600 mg of moist tissue)

Experiment	Lost β-alanine, μM	Surplus formation of N-urea, μM	Surplus formation of alanine μM
1	5.9	5.6	0.6
2	7.2	6.3	1.1
3	3.5	3.0	0.6

The urine for analysis after injection of β-alanine was collected over a period of 48 hours, since a specially conducted experiment showed that the urine which was collected on the third day after injection of β-alanine did not contain this amino acid. In the course of the first 8 days it is difficult to detect any difference in the quantity

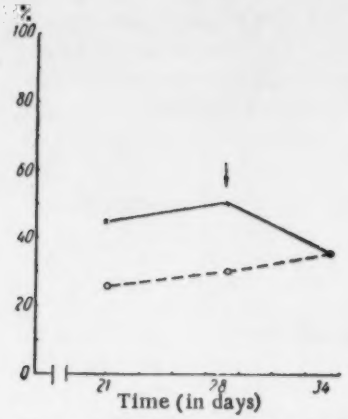

Fig. 2. The effect of INH on β-alanine metabolism in vivo in rats (mean results from Table 2).
— experimental; - - control; ↓ commencement of pyridoxin treatment. Along the ordinate is represented the quantity of β-alanine excreted in the urine as a percentage of the quantity of β-alanine injected

of β-alanine excreted in the urine of the experimental and control animals. On the 2nd day that the animals were kept on their synthetic diet (Table 2) the control rats excreted 23-29% of injected β-alanine in the urine and the experimental (vitamin B_6 deficient) rats – 40-47%, and on the 28th day the figures were 28-33% and 48-55% respectively. Thus vitamin B_6 deficiency increases the quantity of β-alanine excreted in the urine by approximately 20%. After injection of the experimental rats with pyridoxin this excretion of β-alanine falls and reaches the same values as in the control animals (Fig. 2).

Nevertheless the tissues of the experimental rats must evidently contain some quantity of vitamin B_6, since otherwise the animals would have died. A small part of the β-alanine injected into the experimental animals probably continues to take part in the processes of transamination. In addition β-alanine may be concerned in metabolic processes not dependent on pyridoxin enzymes: for example it may act as raw material for the synthesis of dipeptides such as carnosine and anserine [11, 12, 13], it may presumably be a source of formation of pyrimidine bases [10], and possibly it may undergo other conversions.

Attention is drawn to the gradual increase in the quantity of β-alanine excreted by the control rats (Table 2, Fig. 2). A parallel fall is observed in the weight of both the experimental (25-33%) and control animals (18-23%). This is due to the lack of balance of the synthetic diet in respect to a number of factors (besides vitamin B_6) directly or indirectly concerned in the metabolism of β-alanine.

In conclusion we must compare our findings with the experimental results of Pihl and Fritzson [13]. These authors showed that in the course of 5 hours, more than 90% of the labeled carbon of the carboxyl group of β-alanine injected into rats was excreted in the form of CO_2. In other words in 5 hours more than 90% of the injected β-alanine has undergone decomposition. In the control rats in our own experiments, in two days about 25% of the

TABLE 2

The Effect of INH on the Metabolism of β-alanine in Vivo in Rats

Date		2/5/58 start of expts.	2/24/58 after 19 days			3/3/58 after 26 days			3/9/58* after 32 days		
Rat no.		rat wt. (in g)	β-alanine injected A (in mg)	β-alanine excreted B (in mg)	$\frac{B}{A}100$	A (in mg)	B (in mg)	$\frac{B}{A}100$	A (in mg)	B (in mg)	$\frac{B}{A}100$
Experimental	1	170	122	58.0	47.5	122	67.4	55.2	127	52.2	41.1
	2	170**	0	none			none		0	none	
	3	170	122	49.4	40.3	120	57.2	47.8	115	35.1	30.4
	4	170	136	62.1	45.6	132	65.7	49.8	1***	–	–
Control	5	170	141	40.7	28.9	135	44.5	32.9	138	53.2	38.5
	6	170**	0	none		0	none		0	none	
	7	170	143	36.2	25.2	134	37.7	28.2	130	42.1	32.4
	8	170	133	31.4	23.5	–	–	–	130	48.5	37.2

* After 4 days of treatment of the experimental animals with pyridoxin (150 μg per day)
** A blind test was done as a control of the absence of β-alanine from the urine of rats not injected with β-alanine.
*** Rat died.

injected β-alanine was excreted in the urine in an unchanged form, and consequently the decomposition of this amino acid did not in any case exceed 75%, i. e. was considerably less than in the experiments of Pihl and Fritzson. The difference between the results obtained is due to the different quantity of β-alanine injected in the experiments: with Pihl and Fritzson this amounted to 1-1.4 mg per rat, and in our experiments it was about 100 times greater, i. e. it amounted to 120-140 mg.* Summing up, it can be stated that after the parenteral injection of rats with 120-140 mg of β-alanine, the quantity of this aminoacid excreted unchanged in the urine of vitamin B_6 deficient animals is greater than in controls, which gives additional evidence of the participation of pyridoxin enzymes in the metabolism of β-alanine.

In isolated liver tissue the added β-alanine acts as a source for the formation of extra urea and also of α-alanine.

SUMMARY

β-alanine was incubated with chopped liver tissue of rats in oxygen for 160 minutes at 37°C. It was established that a certain part of added β-alanine (about 12%) disappears, causing an excessive production of urea and of a small quantity of β-alanine.

In parenteral administration of β-alanine an increased urinary excretion of this amino acid is observed in B_6-deficient rats in which the process of β-alanine transformation is disturbed.

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* Experiments in which rats were injected with 200 mg of β-alanine and the urea nitrogen of the urine was subsequently analyzed as $N-NH_3$ and $N-NH_2$ were also carried out by A. N. Parshin, T. A. Goriukhina and E. A. Gromyko [5]. However, their published results cannot be used for comparison for the following reasons: these workers injected rats with 200 mg of β-alanine or 31.5 mg $N-NH_2$, and they found in the urine an excess of nitrogen which was considerably greater than this figure, composed of three fractions (N–urea + $N-NH_3$ + $N-NH_2$) totalling from 46.1 to 117.7 (!) (instead of 31.5 mg). Furthermore these workers estimated the β-alanine excreted by the value of the $N-NH_2$, determined in the urine by a nonspecific "copper" method, which was increased five times.

** See English translation.

*** In Russian.

LIPID METABOLISM IN THE LIVER AND THE EFFECT OF GLUTATHIONE ON THIS PROCESS DURING EXCESSIVE INTAKE OF CHOLESTERIN IN THE DIET

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The numerous functions of the liver include its participation in water and lipid metabolism. The synthesis and decomposition of lipids, the conversion of saturated fatty acids into unsaturated and their further oxidation, the synthesis of cholesterin, and many other biochemical processes take place in the liver.

The lipid content of the liver depends on the state of the diet and on the rate of metabolism in the body as a whole. An increase in the lipid content of the liver may be due to general obesity and overeating, emaciation, starvation, poisoning by phosphorus, alcohol, chloroform, arsenic and phloridzin; it arises also in certain infectious diseases (septicemia, typhoid fever, smallpox, scarlet fever and gas gangrene) and during disorders of phosphatide metabolism. The accumulation of lipids in the liver is observed also when excessive cholesterin is taken in the diet. Cholesterin, accumulating in the liver and other organs and tissues, suppresses the oxidation of fatty acids and the formation of phospholipids [3, 5]. The deposition of cholesterin in the intima of arteries leads to the development of atherosclerosis. Experimental atherosclerosis has been produced in rabbits, dogs, fowls, chicks and guinea pigs [1].

Depending on its etiology, fatty infiltration of the liver can be reversed by a suitable diet, by the addition to the food of choline, betaine, methionine, inositol, the lipocaic substance of the pancreas [2], various vitamins (vitamins B_6, C, biotin, pantothenic acid, etc.), hormones, and other preparations.

The aim of our investigation was to study the changes in the total content of cholesterin, lipids, the various fractions of phospholipids (lecithin + cephalin, sphingomyelin) and of water in the liver during experimental atherosclerosis in chicks caused by the addition of 1% of cholesterin to their usual diet. We also investigated the effect of glutathione on these changes. The effect of this last substance was studied in connection with the work of Carey, Vollmer, Zwemer and Spence [5] who found that the subcutaneous injection of glutathione leads to a significant fall in the cholesterin content of the adrenals in guinea pigs.

EXPERIMENTAL METHOD

Investigations were made on 3 groups of 10-day old chicks. In the first group 23 chicks (controls) received sunflower oil (2.5%), with millet gruel and finely chopped green grass; 20 chicks in the second group were given grass and millet gruel to which had been added 1% cholesterin and 2.5% of sunflower oil; 19 chicks of the third group received the same diet as the chicks of the second group and, in addition, they were given daily subcutaneous injections of 1 ml of a 1% solution of glutathione, starting on the 3rd day of the experiment. Eleven days after the start of the experiment the chicks were decapitated. In the chicks receiving cholesterin, at postmortem examination the liver was clearly seen to be stained yellow; in those chicks which had been given cholesterin and glutathione the yellow staining was less clearly marked. The liver was taken out, weighed and dried at 102-105°C to constant weight. The dry residue was ground in a mortar and wrapped up in previously weighed and dried pieces of filter paper, after which the powder was again dried at 102-105°C, weighed and

Composition of the Lipids of the Liver and Weight of Control Chicks (1st Group), of Chicks Receiving Cholesterin (2nd Group) and Receiving Cholesterin and Glutamine Simultaneously (3rd Group)

Type of analysis	Groups		
	1	2	3
Wt. of chicks in g	67,0	61,6	64,5
Wt. of dry liver in g / 100 g wet wt.	4,55	5,25 (+15,4)	5,03 (+10,5)
Wt of dry residue of liver in g / 100 g wet wt.	1,22	1,58 (+29,5)	1,38 (+13,1)
Water content of liver in %	73,1	69,8 (—4,4)	72,3 (—1,1)
Lipid content of raw liver in %			
" " in ether extract	4,70	8,72 (+80,6	6,12 (+30,2)
" " in alcohol extract after extraction with ether	3,20	2,63 (—17,8)	2,64 (—17,5)
Total lipid content	7,90	11,35 (+43,6)	8,76 (+10,9)
Wt of dry residue without lipids in %	19,0	18,85	19,94
Content of cholesterin in liver in %	0,49	3,14 (+541)	1,37 (+179)
" of lipids without cholesterin in %	7,41	8,21 (+10,8)	7,39
" of lecithin + cephalin in %	1,62	1,17 —27,8)	1,25 (—22,8)
" of sphingomyelin in %	1,59	1,25 (—21,4)	1,29 (—18,9)
Coefficient: $\frac{\text{phospholipids}}{\text{cholesterin}}$	6,55	0,77	1,86

Notes: 1. Mean values are shown in the Table. 2. The figures in brackets represent the percentage of increase (+) or of decrease (-) in comparison with the figures for the control group.

placed in a Soxhlet's apparatus, in which it was extracted with ether for 3-5 days. The ether extracts were collected in separate glass receivers, and the powders were dried at first in air and then at 102-105°C, and placed once more in the Soxhlet's apparatus; here they were subjected to prolonged (3-5 days) extraction with alcohol. The weights of the ether and alcohol extracts were estimated by the difference in weight of the powders before and after extraction, and also by the weight of lipids obtained after evaporation of the ether and alcohol and the subsequent drying of the extracts. By this treatment of the tissue, determining the phosphorus content of the alcohol and ether extracts separately, we were able to calculate the content of lecithin + cephalin and of sphingomyelin alone. The neutral lipids, lecithin, cephalin and cholesterin were extracted with ether, and the sphingomyelin and cerebrosides with alcohol (after preliminary treatment with ether). During the course of the experiment the chicks of the 2nd and 3rd groups ate 2.3 g of cholesterin each.

EXPERIMENTAL RESULTS

The results obtained were calculated per dry weight of liver and are shown in the Table.

It is seen from the figures in the Table that the administration of cholesterin with the diet causes an increase in the wet weight of the liver of chicks of 15.4%, and of the dry residue – of 29.5%. The increase in the weight of the liver and of its dry residue is connected with an accumulation of lipids, since the weight of the dry residue of liver without lipids is almost the same in the three groups of chicks (19, 18.85 and 18.94%). The total lipid content in the chicks of the 2nd group was increased by 43.6%, and the ether-soluble fraction by 80.6%. The content of alcohol-soluble lipids fell by 17.8%. The cholesterin content of the liver in the chicks in group 2 was increased 6.4 times in comparison with the controls. In the chicks receiving cholesterin the content of lecithin + cephalin fell by 27.8% and that of sphingomyelin by 22.8%. The ratio of the phospholipid content of the liver to the cholesterin content of the chicks of the 2nd group is 8½ times less than to that of the control group.

In the chicks of the 3rd group, which received cholesterin and glutathione, the weight of dry residue of liver was increased to a lesser degree than in the chicks of the 2nd group which received cholesterin alone (29.5 and 13.1% respectively). The same thing is observed in respect of the total quantity of lipids, the lipids of the ether extract, and the cholesterin. The increase in the content of the latter in the liver of the chicks of this group was only 179% as compared with 541% in the 2nd group. Thus the subcutaneous injection of glutathione leads not only to a fall in the cholesterin content but also prevents the deposition of other lipids in the liver of the experimental chicks. The mechanism of action of glutathione is not yet explained and requires further study. It is possible that other SH- and S–S- containing compounds would have a similar effect.

SUMMARY

Chicks fed for 11 days with finely chopped green grass and millet porridge with 1% cholesterin and 2.5% of sunflower seed oil, developed liver hypertrophy. The content of water in the liver, as well as of the lecithin + cephalin and sphingomyelin decreased, while the total content of the lipids increased. Daily administration of 1 ml of 1% glutathione solution markedly reduced these disturbances.

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MITOGENETIC ANALYSIS OF THE PROTEIN STRUCTURE OF PROTOPLASM

COMMUNICATION I. EXPERIMENTS ON A GELATIN MODEL

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It may be concluded from a number of factors that in living systems the spatial relationships of the molecules are by no means entirely of the nature of stable, large complexes, so-called "structures", due to true chemical bonds.

Equal importance, however, is attached to temporary quasichemical bonds (of the hydrogen bridge or electrovalent bond type, etc.) and their continuous interchange.

In the study of these processes both in vitro and in the protoplasm of living cells, mitogenetic methods open up new prospects.

For this reason we set out to discover by means of the mitogenetic method criteria which would permit estimation of changes in the quasichemical bonds (reappearance or disappearance) in the protoplasm.

However, before embarking on this study on a living object it was necessary to define the limits of sensitivity of the method on a suitable experimental model.

EXPERIMENTAL METHOD

In selecting a test object we decided upon gelatin. According to modern ideas of colloid chemistry a gelatin sol consists of molecules in chain formation.

Interaction between the individual molecules takes place through nonpolar groups [3]. A gelatin sol, heated to 30-40°C, splits up into separate polypeptide chains which, on cooling, recombine to form a gel. Several theories of gel-formation exist. The most widely accepted is the theory of crystallization which was suggested by Levites, Lipatov, Sokolov, Pauli, Meyer, Mark and others.

This theory regards the process of gel-formation as a peculiar form of crystallization – a gradual approximation and orientation of hydrated particles.

In dilute solution gelatin exists in the form of polypeptide chains; these interact through nonpolar groups. The main forces of interaction within the chain are the valent bonds between the amino acid residues.

On cooling the gelatin, hydrogen bridges are formed between the imido (NH) and carbonyl (CO) groups, and also bonds of an electrostatic character, as a result of which the chains become aggregated into two-dimensional flat lattices, the distance between the chains being of the order of 4.5 A.

In the subsequent development of the process of gel-formation the two-dimensional flat lattices form three-dimensional complexes on account of electrovalent bonds which arise in the side chains containing ionogenic groups. The distance between the lattices is of the order of 11.3 A.

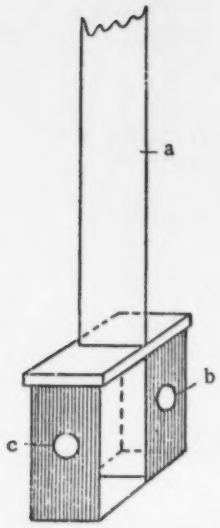

Fig. 1. Quartz chamber. a – China partition, b – quartz window through which the activating ultraviolet light is delivered; c – quartz window centered in front of the collimator of the spectrograph.

In the three-dimensional complexes the bond in the direction of the chain is more closely expressed than are the bonds between the elements in the other two directions, and is due to Van der Waals forces and to hydrogen and electrostatic bonds [3]. These elements are anisometric and form a reticular structure, filled with fluid. Thus, the difference between the sol and the gel consists of the formation in the gel of new and feebly energized bonds.

It is on this condition of the formation of a gelatin gel by labile, feebly energized bonds that the use of gelatin is based as a model to enable the investigation of the possibility of determining the bonds by spectral methods. For this purpose we utilized the mitogenetic method of spectral analysis of selective dispersion, introduced several years ago [1]. As a biological indicator we used an 18-hour culture of the wine yeast Saccharomyces cerevisiae pacca chabli on solid agar.

The effect of irradiation was calculated from the formula $E = I - C / C$, where E is the effect, I the induction and C the control [2].

In order to obtain the selective spectrum of the mitogenetic radiation from the gelatin solution we used a quartz chamber, illustrated in Figure 1. Through the window b in one wall of the chamber the ultraviolet radiation is delivered.

The spectrum is obtained through the window c, in another wall of the chamber, which is divided by a china partition which extends to within 2 mm of the base. This type of construction, with the communicating vessels, permitted the continuation of chain processes underlying the selective dispersion of the mitogenetic radiation, and enabled the effects of scattered "parasitic" light to be excluded.

The investigation was made on a 3% aqueous solution of gelatin.*

The solution was prepared before the experiment in a water bath for 15 minutes, and then was transferred to the chamber, cooled to the required temperature and placed in the apparatus to secure a constant temperature. After the spectrum had been obtained from the sol, the chamber was placed in the refrigerator in order to gelate the gelatin, for 40-60 minutes. The spectrum from the gel was then obtained.

When studying the spectra of the sol and gel, we set out from the hypothesis that in the process of conversion of gelatin from the sol into the gel state, a reorganization of the intermolecular bonds takes place. It was therefore to be expected that certain changes would take place in the spectrum.

We were not concerned to obtain the complete spectrum of the gelatin. We confined ourselves to the study of the $R-C_6H_5$ group – the phenyl group of phenylalanine present in the side-chain – and the $R-\overset{R}{\underset{\backslash}{C}}=O$ or carboxyl group, present in the main peptide chain. These functional groups were of the greatest interest to us since, as pointed out in the scheme described, a hydrogen bond appears during the formation of the two-dimensional lattice between the carbonyl and the imido groups, whereas the formation of the three-dimensional framework is due to electrovalent bonds between the functional groups of the side-chains, one of which is phenylalanine.

EXPERIMENTAL RESULTS

As seen from the Table and Figures 2 and 3, our hypothesis that changes in the intermolecular bonds of the Van der Waals and hydrogen bridge type would be sharply reflected in the spectra of selective dispersion was fully justified.

* The gelatin was obtained from Prof. V. A. Kargin's laboratory (L. Ia. Karpov Institute of Physical Chemistry).

Selective Dispersion Spectra of the Functional Group

$R-\overset{R}{\underset{|}{C}}=O$ and $C-C_6H_5$ in a Sol and Gel of Gelatin

Functional group	Wavelength in A	Av. effect (in %)		No. of expts	
		sol	gel	sol	gel
C=O	1960—1975	72,0	58,0	10	15
C=O	2100—2110	85,0	60,0	10	15
Next to c C=O	1950—1960	1,0	76,0	11	9
The same	1975—1980	1,5	1,2	8	4
» »	2090—2100	1,2	2,0	4	8
» »	2110—2120	1,1	70,0	9	10
C_6H_5	2690—2700	131,0	89,0	3	4
C_6H_5	2700—2710	78,0	94,0	5	5
C_6H_5	2720—2730	95,0	45,0	7	7
Next to c C_6H_5	2710—2720	9,5	73,0	7	8
The same	2730—2740	15,0	91,0	5	4

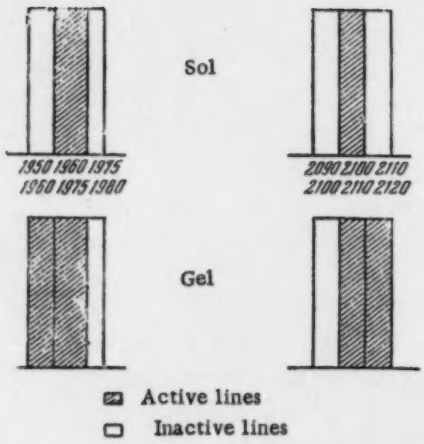

Fig. 2. Spectrum of the functional group $R-RC=O$ in gelatin.

Duration of Exposure of Activity of the Functional Groups

$\begin{matrix} R \\ R \end{matrix}\!\!>\!C=$ and $\begin{matrix} R \\ R \end{matrix}\!\!>\!C_6H_5$

Functional group	Wavelength in A	Exposure	Effectiveness of line	
			sol	gel
C=O	1960-1975	25″	+	+
C=O	2100-2110	15″	+	+
C_6H_5	2690-2700	25″	+	+
C_6H_5	2700-2710	18″	+	0
		30″	0	+
C_6H_5	2720-2730	25″	+	+

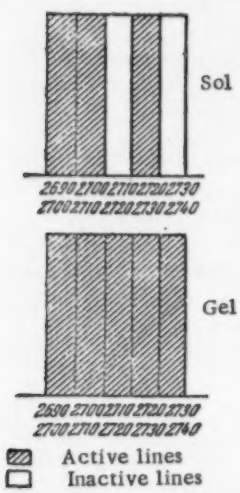

Fig. 3. Spectrum of the phenyl group $R-C_6H_5$ of phenylalanine in gelatin.

The characteristic bands of $R-C=O$ (1960 - 1975 A) and (2100 - - 2110 A), studied with an accuracy of up to 5 A, were surrounded in the sol by unoccupied areas; in the gel these bands were still present and new bands appeared alongside them: in the short-wave side a band at 1950 - - 1960 A, and in the long-wave side – at 2110 - 2120 A (Fig. 2).

According to the hypothesis put forward above, we regard the appearance of these new bands in the gel as an expansion of the bands of the carbonyl group.

The spectrum of this group is represented in the sol by 3 bands at 2690 - 2700 A, 2700 - 2710 A and 2720 - 2730 A, and by unoccupied areas at 2710 - 2720 A and 2730 - 2740 A. In the gel the spectrum is widened and the bands at 2710 - 2720 A and 2730 - 2740 A, inactive in the sol, begin to emit radiation, i. e. the entire area from 2690 - 2740 A becomes active.

There is a conspicuous weakening of the 2nd C_6H_5 band in the gel.

This band is effective in the sol for 18″, and in the gel for 30″.

We cannot give at this juncture a complete discussion of this phenomenon, but we consider it necessary to emphasize the fact itself.

The changes in the spectrum of the phenyl group bear the same character.

The filling up of the vacant interval (2710 - 2720 A) and the addition of a new band in the long-wave side (2730 - 2740 A) may, it appears to us, be interpreted with full justification as a widening of the main bands, and the phenomenon of widening may itself be considered to be a sign of the establishment of intermolecular bonds (Fig. 3).

SUMMARY

By means of mitogenetic spectral analysis it was established that lines characteristic of the functional groups RC= O (carbonyl) and R= C_6H_5 (phenyl) are of different breadth in the sol and gel of gelatin. When the gelatin sol is changed into gel the lines become broader.

Increase in the breadth of lines may be considered as the result of appearance of weak intermolecular connections, joining the peptide chains into two- and three-dimensional latticeworks.

The results of these experiments permit consideration of these data as a successful criterion in the assessment of the degree of connection of peptide chains in the substrate under different cellular conditions.

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MICROBIOLOGY AND IMMUNITY

THE COMPETITION OF ALLERGIC PROCESSES

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The role of interaction between antigens, and in particular of competition between them in the process of the immunological reorganization of the body, has been the subject of fairly extensive literature. This problem was investigated in a most profound and detailed manner by P. F. Zdrodovskii and his co-workers [3], who were studying the possibility, the conditions, and the laws of inhibition of the immunological activity of the body. There are indications of the inhibitory effect of sensitization of the body on the production of antibodies – agglutinins and antitoxins [5, 7, 1].

The role of interaction between allergens in the development of a state of increased sensitivity of the body has received far less study. In this problem, which is of theoretical and practical importance, the only aspect which has been investigated is the possibility of increasing allergy by the influence of nonspecific sensitization, mainly during infectious diseases.

The possibility of suppression of development of increased sensitivity of the body as a result of competition between allergic processes has been almost completely ignored. The literature on this subject is extremely limited. Lewis [8], for instance, showed that it was possible to inhibit the development of sensitization to one protein by the simultaneous or subsequent (24 hours after sensitization) injection of a large dose of another protein. However, experiments by A. M. Gardash'ian [2] showed the possibility of development of anaphylaxis to both allergens after sensitization of guinea pigs with a mixture of horse and rabbit sera in the most widely different proportions (from 1:100 to 1:20,000).

The aim of the present investigation was to ascertain the possibility of and the conditions for inhibition of the development of the allergic reorganization of the body as a result of competition between the allergens.

EXPERIMENTAL METHOD AND RESULTS

The experiments were performed on that classic and thoroughly studied model – anaphylactic shock in guinea pigs (initial weight 250 - 280 g), sensitized by a single subcutaneous injection of different doses of horse and ox serum, either simultaneously or by injection of the allergens at different intervals of time. From 21-24 days after injection of the second allergen, an assaulting injection of ox or horse serum was given (0.25-1 ml, intracardiac injection). Under these conditions the main criterion of the degree of allergic reorganization of the animal was the depth of the anaphylactic shock. As is known, the anaphylactic shock resulting from the use of this method is generally fatal.

In the first experiment simultaneous sensitization of the animals with both allergens in different doses was carried out (Table 1).

As the results given in Table 1 show, the simultaneous sensitization with two antigens, in which one is greatly in excess of the other, leads to a less profound allergic reorganization in respect to the second allergen (in 3 of 6 guinea pigs, after an assaulting injection of 0.25 ml of ox serum only slight manifestations of anaphylactic shock were observed). The results obtained confirm the experimental findings of V. I. Ioffe and his

TABLE 1

Development of Anaphylaxis to Ox Serum After Simultaneous Sensitization with Two Allergens

Guinea pigs	No. of animals	Sensitizing dose of serum		No. surviving shock
		horse	ox	
Experimental	6	0,01	0,01	0
	6	0,1	0,01	0
	6	1,0	0,01	3
Control	3	0,01	—	0
	3	1,0	—	0
	3	—	0,01	0

co-workers [4], who demonstrated the possibility of suppression of the development of sensitization to one of the components of a combined allergen when the other was present in considerable excess.

In the next experiments we studied the development of sensitization after injection of allergens at different intervals. In these experiments sensitization to the second allergen (ox serum) was produced both at the onset of the allergic reorganization (after 72 hours) and at its height (after 7-14 days).

The guinea pigs were sensitized by the subcutaneous injection of 0.01-1.0 ml of horse serum. After 3-7 or 14 days, sensitization of the experimental animals with 0.01 ml of ox serum was carried out. The assaulting injection of ox serum was given 21-24 days after sensitization with the second allergen. The results of the experiments are shown in Table 2.

From these experimental results it can be concluded that suppression of the development of sensitization to ox serum is observed when the sensitizing dose of this serum is injected at the height of the allergic reorganization of the animal to the other antigen (from 7-14 days after sensitization).

It must be pointed out that of the 6 guinea pigs sensitized to ox serum 7 days after the injection of horse serum, and not developing fatal shock after the assaulting injection of ox serum, in three animals the manifestations of shock were extremely slight. After sensitization of the animal with equal doses of each allergen at intervals of 7 days, some degree of weakening in the intensity of the allergic reorganization to the second allergen was also observed.

Animals surviving an assaulting injection of one of the allergens (usually ox serum) were given an assaulting injection of the second allergen. It was shown in this way that the animals which had survived anaphylactic shock appeared to be desensitized to injection of the second allergen if it was injected within a few hours after survival from shock. Under these conditions the degree of desensitization was inversely proportional to the severity of the shock which the animal had survived. Twenty-four hours afterwards the sensitization was usually completely restored.

It can thus be concluded from the results obtained that at the height of development of sensitization the animal is observed to be relatively refractory to the injection of a nonspecific allergen. This refractoriness is not, however, found in all animals by the method which we used, and is less marked than in inhibition of

TABLE 2

The Development of Anaphylaxis After Successive Sensitization by Two Allergens After Different Intervals of Time

Guinea pigs	No. of animals	Intervals between sensitizations	Sensitizing dose of serum		No. surviving shock
			horse	ox	
Experimental	5	72 hrs	1,0	0,01	0
	10	7 days	1,0	0,01	6
	5	7 »	0,01	0,01	2
	5	14 »	1,0	0,01	3
Control	5	—	1,0	—	0
	5	—	—	0,01	0

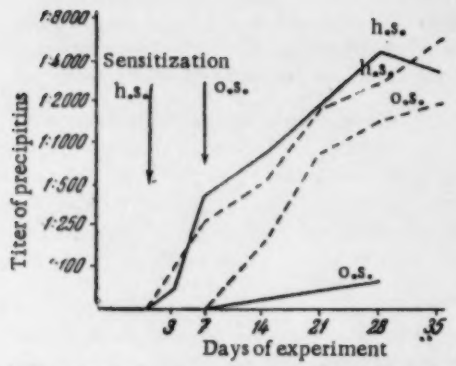

Fig. 1. Average titers of precipitins to horse and ox protein.
— experimental group; ---control groups; h. s.— — horse serum; o. s. — ox serum.

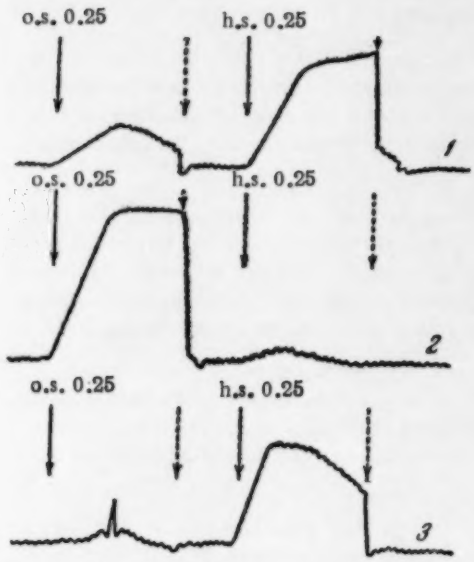

Fig. 2. Reaction of the isolated intestine of sensitized guinea pigs (experiment 30 days after sensitization).
Experimental group: 1 — sensitization to 1.0 ml of horse and 0.01 ml of ox sera at intervals of 7 days. Control group: 2 — sensitization to 0.01 ml of ox serum; 3 — sensitization to 1.0 ml of horse serum; h. s. — horse serum; o. s. — ox serum; ↓ rinsing with Ringer-Locke solution.

antibody formation. This is probably due to the greater sensitivity of the guinea pig to an anaphylactic reaction.

The development of serum anaphylaxis is known to be accompanied by the production of precipitins to the allergen injected. It was considered to be of interest to investigate whether inhibition of antibody formation is observed under these conditions. For this purpose blood was taken from all the experimental animals (20 guinea pigs) 3, 7, 14, 21 and 28 days after the first sensitization, and the titer of the precipitins in the serum was determined.

It was found that 3 days after sensitization the titer of the precipitins to horse serum varied from 0 to 1:100, 7 days after — from 1:100 to 1:800, increasing on the 21st-28th day to 1:1000-1:8000. The injection of the second allergen did not affect the precipitin titer. The antibody titer of the control animals also increased in a similar manner.

The precipitin titer to the second allergen — ox serum, injected into the experimental animals 7 and 14 days after sensitization to horse serum — altered in a different manner. In this case, 21 days after the injection of ox serum in not one of the 20 animals did the precipitin titer exceed 1:100, and in 4 guinea pigs precipitins were found only in titers less than 1:10. In control animals, receiving only 0.01 ml of ox serum (5 animals) the antibody titers at this time varied from 1:800 to 1:2000 (Fig. 1).

From the results obtained it may be concluded that inhibition of the allergic reorganization of the animal is accompanied also by inhibition of immunogenesis, and also that this latter is more marked and is observed in all the experimental animals. No direct quantitative relationship between the degree of sensitivity of the animal and the precipitin titer in the blood of the animal could be observed.

In the pathogenesis of the anaphylactic reaction, spasm of smooth musculature plays a highly important part [11 and others], and the sensitivity of this tissue to the allergen increases sharply in the process of sensitization [8, 10]. This change in the sensitivity of the smooth musculature is specific for the allergic reorganization.

Experiments were carried out to ascertain the effect of competition between allergens on the development of increased reactivity of the smooth musculature. In the literature no investigations of this sort have been reported. The experiments were carried out on an isolated segment of intestine, from 24-30 days after sensitization to the second allergen, by the generally accepted method of Schultz and Dale [6].

It was shown by these experiments that after sensitization to the allergens at an interval of 7 days, the reaction of the smooth musculature was considerably more marked to the first allergen – horse serum (Fig. 2), whereas in a comparison of the kymograms from the corresponding control animals this difference was not observed. However, the reaction of the isolated intestine to the second allergen was nevertheless somewhat more marked than in unsensitized animals, which suggests only a reduction in, rather than a complete absence of, allergic reorganization of the smooth musculature to the second allergen.

Altogether 19 guinea pigs and corresponding controls took part in the experiment.

Thus the results obtained demonstrate that the laws of excitation and inhibition, discovered by N. E. Vvedenskii and I. P. Pavlov for nervous processes, and confirmed by P. F. Zdrodovskii and his co-workers for the immunological reactivity of the animal body (antibody formation), are also true for the allergic reorganization of the body (anaphylaxis). In this case also, at the height of the allergic reorganization the animal is relatively refractory to the action of other stimuli. This refractoriness also extends to the function of antibody formation (precipitins), which emphasizes once more the close interconnection between these processes. Investigations carried out on isolated organs (segments of the small intestine) have shown that the reduction in the degree of allergic reorganization is also accompanied by a reduction in the reaction of the smooth musculature to the corresponding allergen, a feature which was observed in all the experiments carried out.

The results obtained call for a fresh reappraisal of the special features of the course of secondary infections, developing in association with an underlying disease, always accompanied by an allergic reorganization of the body.

It is essential to bear these findings in mind when composing rational schemes of combined immunization, since the allergic reorganization of the body plays an essential role in the vaccinal process.

SUMMARY

This work was conducted on guinea pigs sensitized by various doses of horse and ox sera simultaneously or at various intervals between the administration of the allergens (on the 3rd, 7th, 14th and 21st day). The allergic reorganization was judged by the death of the animals in injecting the booster dose of one of the allergens, the value of the antibodies – precipitins for them, as well as the reaction of the isolated intestinal portion to the allergens. It was demonstrated that at the peak of the allergic reorganization (in 7-14 days) the body is relatively refractory to the sensitization by other allergens, which is demonstrated by the survival of more than 50% of the animals injected with a booster dose (with the 100% mortality rate of the control animals), by the considerable depression in the production of precipitins, and decreased reaction of the smooth muscles to the second allergen.

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THE PLASMOCYTIC REACTION AND IMMUNOLOGICAL LAWS

COMMUNICATION II. IMMUNOLOGICAL INHIBITION DURING IMMUNIZATION OF RABBITS WITH TETANUS TOXOID

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In a previous paper [1] we showed that the characteristic cytological changes accompanying the immunological process and called, not very accurately, the plasmocytic reaction, fully obey the laws of revaccination and immunological inhibition. The applicability of the law of revaccination to the cytological changes was proved for corpuscular and soluble antigens, but the cytological basis of immunological inhibition was studied for corpuscular antigen only. Furthermore it is known that the development of immunological inhibition under the influence of corpuscular antigen differs essentially from the inhibition which arises during the action of soluble antigen (toxoid). In addition, when comparing the periods of development of the cytological changes on the one hand, and the increase in the blood antitoxin level on the other hand, in our first investigations we found a fundamental time lag between the two processes, measuring 20-25 days in primary immunization and 6-8 days in revaccination. Such a marked discrepancy in the timing of these processes merits serious attention in evaluating the role of the so-called "plasmocytic reaction" in immunogenesis, and for this reason the discovery of the causes of this discrepancy requires further investigation.

In view of what has been stated above, under the direction of P. F. Zdrodovskii we undertook a detailed study of the inhibitory process in rabbits caused by the repeated injection of a soluble antigen (toxoid), with separate determinations of antitoxin in the regional and distant lymphatic nodes, the spleen and the blood, and at the same time carrying out a cytological examination of these organs listed.

EXPERIMENTAL METHOD

The experiments were carried out on 52 rabbits which, 6 months previously, had received a single injection of 1 cc of crude tetanus toxoid subcutaneously in the lower part of the hind limb.

The rabbits prepared in this way were subjected to repeated injections of 1 cc of the same antigen in the same area at intervals of 5 days. In a period of 72 days 13 injections were given, with an interval of 10 days after the 7th injection of toxoid.

On alternate days three rabbits were killed by exsanguination, by a method described in the previous paper. After complete removal of blood from the organs to be examined, smears and saline extracts were prepared from them. On the same day blood was taken from the veins of the ear in these rabbits.

Preparation of the extracts. After exsanguination of the animal the right and left lymphatic nodes and the spleen were removed and placed in sterile Petri dishes. Then the above-mentioned organs were weighed on torsion scales (the lymphatic nodes completely, the spleen in part). The weighed tissue was placed in glass homogenizers* shaped like test tubes, and physiological saline was added to the tissue. Maceration of the tissue

* For a detailed account of the technic of homogenization, see Umbreit [2].

TABLE 1

Individual Results of Cell Counts of the Plasmocytic Series in Rabbits in Response to the 1st and 2nd Revaccination

Serial no. of revaccination	Day after revaccination	Rabbit no.	Results of count: right lymphatic node	left lymphatic node	spleen
1st revaccination	3rd	1	150/5	19/1	19/8
		2	111/5	17/0	—
		3	138/4	17/0	8/16
		Mean . . .	133/5	18/0	13/12
	5th	4	11/12	9/10	1/18
		5	13/2	14/2	6/18
		6	37/19	11/0	4/16
		Mean . . .	20/11	11/4	4/17
2nd revaccination	3rd	7	164/64	20/0	22/11
		8	137/8	28/0	—
		9	147/4	22/18	7/15
		Mean . .	149/25	23/6	14/13
	5th	10	20/30	8/16	18/68
		11	72/2	16/4	2/36
		12	34/18	6/4	0/13
		Mean . .	42/17	10/8	7/39

Note: The numerator indicates the number of young forms of the plasmocytic series; the denominator – the number of mature plasma cells.

was carried out by means of a pestle which was made to revolve in the tube by an electric motor. The homogeneous mass thus obtained was transferred to sterile tubes, and the homogenate remaining on the walls of the homogenizer tube was twice washed with small quantities of the same solution in order to obtain the required volume. The main dilutions of the organs which were used were extracts diluted 5-10 times, depending on the size of the organ and the period of investigation.

The prepared extracts were placed in the refrigerator, and next day titration of the antitoxin was carried out separately for each organ and the serum. Experience showed that keeping the homogenate for up to 12 days (longer periods of keeping were not tested) does not lower the antitoxin titer. The antitoxin was determined by K. T. Khaliapina's method [3].

Preparation of smears and cytological analysis. The cytological picture of the popliteal lymphatic nodes and the spleen were studied by the smear method. For this purpose the organs were cut up with pointed scissors, and smears were taken from them by careful contact of the cut surface to the glass slide. From 4-6 of these preparations were made, and each smear was taken from a freshly cut surface. The smears were fixed in methyl

alcohol and stained with azure II – eosin. During the examination of the smears, for counting the cells we took in succession all the fields of vision which had a uniform, continuous distribution of cells. In this way, with a magnification of 90 × 7, in each field of vision from 100-200 cells were counted. Lymphoblasts and plasma cells, mature and immature, were counted; the total number of these forms in 50 fields of vision was determined, with meticulous observation of the above conditions of selection of the fields. The results for each period are shown as the average of the cell counts in three rabbits. Examples of the individual figures are shown in Table 1.

As it follows from Table 1, the individual variations in the limits of one period were moderate.

EXPERIMENTAL RESULTS

The investigations established a high content of antitoxin in the regional node, and only on the 3rd day (after 48 hours) after the first revaccination the antitoxin titer was equal to 3 units, reaching a maximum of 13 units on the 5th day. Later, in spite of continuing injections of antigen, the antitoxin level fell, showing from time to time increases in the titers, alternating with new falls, in association with individual variations in the production of antitoxin by the animals.

The same relationships were found in the blood antitoxin curve – the only difference was in the delay in the rise of the antitoxin titer in the first days after injection of antigen. In this case the maximum titer (16.6 antitoxin units) was observed only on the 11th day, after which a gradual fall took place in the antitoxin level.

The lymphatic node of the opposite limb and the spleen hardly participated at all in the production of antitoxin throughout the injection period. The results of this experiment are summarized in Figure 1.

We may mention in passing that weighing of the lymphatic nodes showed a regular increase in the regional nodes to 1½ -2 times that of the lymphatic nodes of the opposite limb.

The results of the cytological investigation of the lymphatic nodes are shown in Figure 2.

As in the previous investigation the main cytological changes consisted of the accumulation of large basophilic cells of a lymphoid type – lymphoblasts, also known as "plasmoblasts" in cases where such an accumulation precedes an accumulation of plasma cells. Since the variations in the content of the latter cells were of quite a different character in our experiments from the variations in the content of young lymphoid forms, these two groups of cells were counted separately. In Figure 2 the variations in the content of each are shown by separate curves.

As shown in Figure 2, each of the first three revaccinations caused a rapid and transient change in the cell composition of the regional lymphatic node, in the form of an accumulation of young lymphoid cells, with a maximum on the 3rd day and a fall on the 5th day. At this period the intensity of each new rise increased, but the depth of the fall gradually diminished. The response to the 4th revaccination was somewhat weaker than that to the 3rd revaccination, and there was no response at all to the 5th revaccination, since in place of the usual increase there was a continued fall in the number of basophilic lymphoblasts. The content of these cells remained at a figure of 65-85, and was practically unchanged after the 6th and 7th revaccinations. After

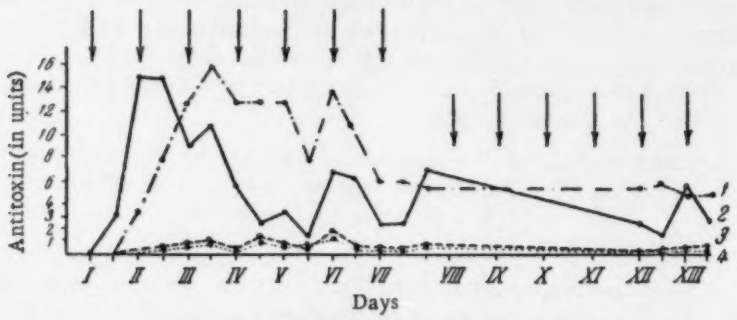

Fig. 1. Accumulation of antitoxin in the blood and organs of the rabbit.
1 – In the blood; 2 – in the right lymphatic node; 3 – the same on the left;
4 – in the spleen. I-XIII – serial numbers of revaccinations.

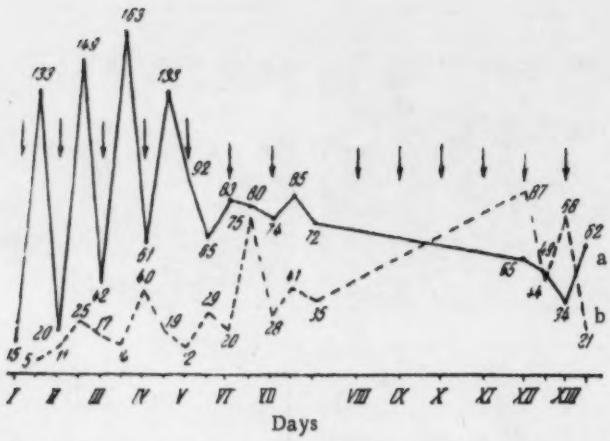

Fig. 2. The trend of the cytological changes after repeated injections of tetanus toxoid (regional node).
a – Immature plasma cells; b – mature plasma cells. I-XIII – serial numbers of revaccinations.

5 more revaccinations, in response to the 12th and 13th injection of antigen, there was no essential change in the content of young basophilic forms.

So far as the adult plasma cells are concerned, their content was low in the first half of the course of revaccinations and rose in the second half of the course. For example, if their average content for the period covering the first 5 revaccinations was 19, in the period between the 6th and 13th revaccinations it was 44.

The character of the quantitative variations in the plasma cells was without that strict regularity which was found in the trend of the young forms (see Fig. 2), and the difference in the individual variations in their content was more significant.

The cytological changes in the distant lymphatic node and also in the spleen throughout the whole course of revaccinations were much less marked. For instance, the average accumulation of basophilic lymphoblasts did not exceed 33 in the lymphatic node on the opposite side, and 20 in the spleen.

After repeated injections of toxoid into prepared rabbits, they develop in their regional lymphatic nodes cytological changes characterized by the accumulation of large cells of lymphoid type with a markedly basophilic protoplasm (lymphoblasts, or plasmoblasts) (see Fig. 3, I, a). Serological investigation of extracts of these nodes shows even at early stages the accumulation of large quantities of antitoxin.

The preferential accumulation of antibodies in the regional lymphatic node was first found by McMaster [8, 9] and Hudack [8], and was confirmed by Ehrich and Harris [4, 5], Harris and Harris [6, 7] and by L. N. Fontalin.*

The frequent repetition of revaccination with toxoid causes development in rabbits of a state of immunological inhibition, which is shown serologically by cessation of the increase in the antitoxin content of the regional node, and by its subsequent fall, and morphologically by absence of the characteristic increase in the number of lymphoblasts in the node.

In reproducing the phenomenon of immunological inhibition we started from the investigations of K. T. Khaliapina, who made a detailed study of this feature in P. F. Zdrodovskii's laboratory.

However, when using a single preparatory inoculation of crude toxoid, we did not achieve such a high level of reactivity as took place in K. T. Khaliapina's experiments, in which two preparatory inoculations were given. The consequence of this variation of K. T. Khaliapina's scheme was a delayed and not-so-sharp fall

* The findings were reported at an extended conference of the Institute of Normal and Pathological Physiology.

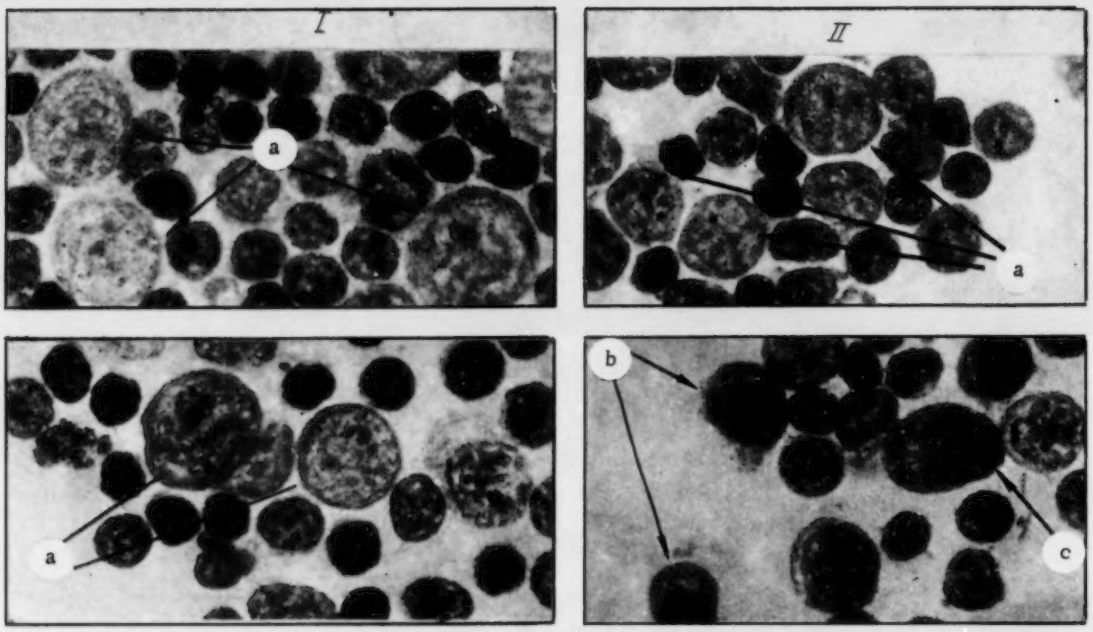

Fig. 3. Smears from the regional lymphatic nodes of rabbits on the 3rd day after the 2nd revaccination (I) and after the 4th revaccination (II).
a – Basophilic lymphoblasts (plasmoblasts); b – immature plasma cells; c – mature plasma cells. Microphotograph, magnification 1000 x.

in the titers of antitoxin in the blood in the inhibitory phase. Even in this less profound inhibitory phase however, the suppression of immunological activity of the node was very significant.

Analysis of the findings reveals the following distinctive features. In the first place, by comparing the times of development of the cytological changes with the trends of the antitoxin level in the regional glands, the complete synchrony of the two processes can be clearly seen. For instance, after the first revaccination (3rd day) a marked lymphoblastic reaction (133) takes place, accompanied by a considerable accumulation of antitoxin (3 units). In this connection the discrepancy previously pointed out between the appearance of the cytological changes in the node and the accumulation of antitoxin in the blood during revaccination is fully explained. The delay in the accumulation of antitoxin in the blood in this case is entirely due to the holding up of antitoxin at the place of its formation, i. e., in the regional node.

In the course of the 2nd and 3rd revaccination the titers of antitoxin in the node are high; the morphological reaction to these revaccinations is very clear. At the time of the 4th revaccination a fall in the antitoxin titers began to be observed. At the same time the first signs appeared of a change in the morphological reaction, shown by the fact that in the interval between revaccinations the morphological picture does not succeed in returning to normal, and the response to the 4th revaccination is weaker than that to the 3rd revaccination. Subsequent revaccinations give a picture of complete inhibition, both morphologically and serologically.

In the second place, the strictly local character of the effect of revaccination is shown with great clarity. In all the rabbits immunized, irrespective of the number of revaccinations, the antitoxin content was highest in the regional node.

The lymphatic node in the opposite limb and the spleen hardly take any part in the production of antitoxin. The morphological observations are in full agreement with the serological findings. The cytological changes, so clear in the regional node, could hardly be detected in the node on the opposite side or in the spleen

The impression is created that the required level of antitoxin in the blood is ensured by the functioning of one regional lymphatic node alone.

This impression is in agreement with the findings of several other workers. Similar results, for instance, were obtained by Ehrich and Harris [5], and by Harris [6]. By extirpation of the regional node or spleen (depending on the mode of injection of antigen), Stavitsky [13] was able to bring about a sharp fall in the effect of revaccination, from which it could be concluded that the synthesis of antitoxin was local in character. Finally, Oakley [see 11], using transplantates, estimated that 1 g of tissue taken from the site of injection of the antigen produces 800 times more antitoxin than the same weight of tissue taken from any other part of the body.

Thus the production of antitoxin is effected mainly in the regional lymphatic node; more distant nodes play hardly any part in this process, despite 13 successive revaccinations. In this respect the immunological inhibition caused by a soluble antigen differs essentially from the inhibition caused by corpuscular antigens. In the latter case, as was pointed out in the previous investigation, repeated revaccinations led to the successive inclusion in the immunizing process of the more distant lymphatic nodes and the spleen. These distinctive features of the process of inhibition during the action of different antigens is of considerable theoretical interest.

Finally, the last special feature of the process of inhibition during the action of tetanus toxoid consists of the character of the cytological changes in the regional lymphatic nodes. As was pointed out earlier, in primary immunization as in revaccination, the main cytological changes are produced in the field of the young forms of the plasmocytic series – plasmoblasts. The content of adult plasma cells was very inconstant and small. The fall in the number of plasmoblasts, taking place after the rise, was not accompanied by any essential accumulation of plasma cells.

For this reason we have to think either of a reverse development of this reaction or, which is more likely, of its termination in ordinary lymphocytes. However, as the number of revaccinations increases, and the resulting state of inhibition becomes more profound, accumulation of adult plasma cells and all stages of their maturation are observed increasingly often. In these cases the whole series of transitional forms connecting the lymphoblasts to the plasma cell can readily be seen (Fig. 3, II, b, c).

On the other hand, many of the basophilic lymphoblasts, preserving their morphology, did not attain such a large size as we had seen previously. Thus, if after the first revaccinations the diameter of the large basophilic lymphoblasts was 18-20 μ (Fig. 3, I, a), then in the stage of inhibition, the dimensions of the lymphocytes were reduced to 10-12 μ (Fig. 3, II, a).

Thus with the fall in the function of the lymphatic node there was a corresponding reduction in the size of the lymphoblasts and the presence of a considerable number of plasma cells, adult and immature.

SUMMARY

This investigation was devoted to the study of the inhibitory process in rabbits caused by repeated administration of tetanus toxoid. Investigations demonstrated that cytological changes develop in the regional lymphatic nodes of rabbits on repeated injection of the toxoid in sensitized animals. These changes are characterized by the accumulation of large cells of lymphoid type with a basophilic protoplasm (lymphoblasts or plasmoblasts). The serological examination of the extracts of these nodes shows the accumulation of large amounts of the antitoxin even in the early periods.

Numerous repeated revaccination with the toxoid causes a condition of immunological inhibition in rabbits, which is serologically manifested by the cessation of the rise of the antitoxin concentration in the regional nodes and later in its drop.

Morphologically this inhibition is manifested in the absence of the characteristic increase of the lymphoblasts in the lymph nodes. The effect of revaccination was strictly local. The content of antitoxin in the regional lymphatic nodes was low irrespective of the number of revaccinations.

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THE SPECIFICITY OF THE HUMORAL FACTORS IN TRANSPLANTATIONAL IMMUNITY

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Problems concerned with the part played by humoral factors of the recipient in transplantational immunity during homografting are of great importance to the discovery of the mechanisms of tissue incompatibility.

During recent years papers have appeared in the literature demonstrating the formation of immune antibodies (hemagglutinins) in the recipient after homografting of dogs' kidneys [6, 7] and of the skin of mice [3, 5]. However, the properties of the antibodies formed during homografting have received little study. According to some workers, the changes in the agglutinating properties of the recipients' serum during homografting may even be of the nature of nonspecific humoral changes [4]. In previous investigations [1, 2] we showed that in the majority of rabbits after homografting of skin immune antibodies are formed, whereas after autotransplantation no antibodies appeared.

The present investigation is devoted to the study of the specificity of the antibodies formed during homotransplantation of the skin in rabbits. We carried out serological investigations on the sera of 19 recipient rabbits containing immune antibodies after homotransplantation.

EXPERIMENTAL METHOD

The properties of the immune sera were studied by means of the hemagglutination and adsorption reactions. The hemagglutination reaction was performed at room temperature, and in a volume of 2 drops in a tube, with subsequent centrifugation for 1 minute at 1500 rpm. The results were read according to the character of the agglutinate. Adsorption was carried out at room temperature with a precipitate of triple-washed red cells in a proportion of 1:1. The serum was first diluted half-and-half with physiological saline.

EXPERIMENTAL RESULTS

Investigation of the rabbit sera before and after grafting with chick's and sheep's red cells showed that the sera of recipients after homotransplantation had not altered their agglutinating properties in relation to heterogenic red cells. The sera of the recipients acquired the property of agglutinating only the red cells of rabbits, i. e. they had the nature of immune iso-antibodies. Immune sera from the recipients were tested with red cells of a large number (60) of the same rabbits.

Table 1 shows the results of the investigation by means of the hemagglutination reaction of the sera of 15 recipient rabbits with red cells of 25 rabbits. In the vertical column on the left are placed the identity numbers of the recipient rabbits whose sera were being examined. In the top horizontal column are shown the identity numbers of the rabbits with whose red cells the examination of the sera was being carried out. A positive hemagglutination reaction is represented as +, a negative -, and doubtful results as ±. The results of the test of the serum with the red cells of the donor and recipient are marked with a ring.

TABLE 1

Antigenic Differentiation of the Tissues of the Rabbits

Category of serum of recipient rabbits	Sera of recipients	Groups of red cells																								
		1st							2nd								3rd			4th						
		Rabbits from which thre red cells were taken																								
		866	407	786	643	701	510	292	1236	71	818	177	77	442	813	333	811	812	756	46	14	365	1717	603	829	67
	866	−	−	−	−	−	−	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+
	510	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I	407	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	292	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
	77	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
	1236	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
II	177	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	+		+	+	+	+	+	+	+
	71	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	+	+		+	+	+	+	+	+	+
	812	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+
III	811	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−		+	+	+	+	+	+	+
	756	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+
	818	+	+	+	+	+	+	+	+	+	−	+	−	−	+	+	+	+	−	−	−	+	+	+	+	+
	442	+	+	+	+	+	+	+	+	+	±	+	±	−	+	+	+	+	−	−	+	+	+	+	+	+
IV	1717	+	+	−			+	−	+	−	−	+		−	+		−	+	−	+	+		−	+	+	+
	786	+	+	−	−	+	+	−	+	−	+	+	−	−	+	+	−	+	−	+	−	−	−	+	+	+

TABLE 2

Properties of the Hemagglutinins of Rabbit No. 1236 after Homotransplantation of Skin

No. of rabbits whose red cells were used for adsorption	No. of rabbits whose red cells were used in agglutination	Dilution of serum						Control red cells
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	
14	1717	+	—	—	—	—	—	—
	812	+	—	—	—	—	—	—
	14	+	—	—	—	—	—	—
	756	±	—	—	—	—	—	—
866	1717	++	++	++	++	+(+)	+	
	812	++	++	++	++	+	—	
	14	++	++	++	+	+	—	—
	756	++	++	++	+(+)	+	—	—
756	1717	+	±	—	—	—	—	—
	812	+	—	—	—	—	—	—
	14	±	—	—	—	—	—	—
	756	—	—	—	—	—	—	—
Serum before adsorption	1717	++	++	++	++	+(+)	+	—
	812	++	++	++	++	+	—	—
	14	++	++	++	+	+	—	—
	756	++	++	++	+(+)	+	—	—
	866	—	—	—	—	—	—	—
	786	—	—	—	—	—	—	—

The serum of each recipient rabbit always gave a positive reaction with the red cells of its donor, but did not agglutinate its own red cells. In addition the recipients' sera agglutinated the red cells of other rabbits, but not all with which they were tested. Thus the serum of recipient No. 1236 agglutinated, besides the red cells of its donor (rabbit No. 1717), also the red cells of rabbits Nos. 67, 756, 46, 812, 14, 811, 829, 603, 365 and 333. The ability of the recipient's serum to agglutinate the red cells of the rabbits listed evidently developed as a result of the presence in these rabbits of common antigens with the donor that were absent from the recipient. It is clear that it is only to this form of antigen in the donor that antibodies could appear in the recipient.

At the same time the recipient's serum did not agglutinate the red cells of rabbits Nos. 866, 786, 442, 407, 818, 510, 813, 77 and 71. The negative reaction was evidently the result of the fact that the rabbits listed had no common antigens with the donor against which antibodies could appear in the recipient. On the basis of their similarity of behavior in relation to the sera of the 15 recipients, the rabbits' red cells may be arranged in several groups.

In the 1st group we placed rabbits Nos. 866, 407, 786, 643, 701, 510 and 292, whose red cells were not as a rule agglutinated by the immune sera of 8 recipient rabbits (Nos. 866, 510, 407, 292, 77, 1236, 177 and 71). However, they gave a positive reaction with the sera of 7 other recipient rabbits (Nos. 812, 811, 756, 818, 442, 1717 and 786). Despite their great similarity, there were also differences between the rabbits of this group. Rabbits Nos. 866 and 407, for instance, differed from the others by the fact that their red cells gave a positive reaction with the serum of recipient rabbit No. 292. Rabbits Nos. 786, 643 and 292 differed from the others by the fact that their red cells were not agglutinated by sera of recipient rabbits Nos. 1717 and 786. At the same time rabbit No. 292 differed from the other rabbits mentioned above by the fact that the sera of recipient rabbits Nos. 866 and 71 agglutinated its red cells.

In the 2nd group we included rabbits Nos. 1236, 71, 818, 177, 77, 442, 813 and 333. The red cells of these rabbits as a rule were not agglutinated by the serum of the 4 recipient rabbits Nos. 77, 1236, 177 and 71.

The sera of the other recipients agglutinated the red cells of these rabbits. Among the rabbits of the second group, as among the animals of the preceding group, despite their similarity, differences were also to be found. For instance rabbit No. 333 differed from the others by the fact that its red cells were agglutinated by the serum of recipient rabbit No. 1236. Rabbit No. 818 differed by the fact that its red cells were agglutinated by the serum of recipient rabbit No. 177. The red cells of rabbits Nos. 77 and 442, which were very similar, behaved differently to the serum of rabbit No. 866 and so on. In a similar manner we distinguished a 3rd and 4th group of rabbits.

Consequently in each group we included rabbits whose red cells behaved in a similar manner in relation to the immune sera of the 15 recipient rabbits, but often there were differences between them. In this way the differences between the rabbits within one group were smaller than those between rabbits belonging to different groups.

The properties of the sera of the recipient rabbits in relation to the red cells of different rabbits were also characterized by features of similarity and dissimilarity. On the basis of the similarity of the properties of the sera in relation to red cells, they could be seen to fall into several categories. In category I were placed recipient rabbits Nos. 866, 510, 407 and 292, whose sera agglutinated the red cells of rabbits of the 2nd, 3rd and 4th groups. However, the sera of the recipients in category I did not agglutinate the red cells of the rabbits in the 1st group. This evidently was because the recipients themselves were members of the 1st group of rabbits. The similarity of the properties of the sera of the recipient rabbits in category I was probably also due to the fact that their donors were rabbits Nos. 67, 46 and 603, which were very similar in an antigenic respect and belonged, as is seen from Table 1, to group 4 of animals. The red cells of one donor (rabbit No. 818), it is true, belonged to the 2nd group. In spite of their great similarity, between the sera of the recipients in category I there were also differences. For instance, the serum of recipient No. 292, in contrast to the others, agglutinated the red cells of rabbits Nos. 866 and 407. The serum of rabbits No. 866 in turn, did not agglutinate the red cells of rabbits Nos. 77 and 813. The sera of recipient rabbits Nos. 510 and 407 showed no differences in relation to the red cells of the 25 rabbits, but when tested with red cells of a larger number of rabbits it was possible to establish differences between them in relation to the red cells of 2 groups of rabbits.

In category II were included recipient rabbits Nos. 77, 1236, 177 and 71, whose sera agglutinated the red cells of rabbits of the 3rd and 4th groups. However, they did not agglutinate the red cells of rabbits of the 1st and 2nd groups. This was evidently because the recipients themselves belonged to the 2nd group. They did not agglutinate the red cells of rabbits in group 1, probably because the rabbits of groups 1 and 2 had certain common antigens. The similarity of the properties of the sera of recipient rabbits in category II was also demonstrated by the fact that their donors (rabbits Nos. 14, 1717 and 829) had a great antigenic resemblance and they belonged to group 4. The red cells of one donor (rabbit No. 811) belonged to group 3. In spite of the fact that the sera of the category II recipients showed great similarity, there were also certain well-defined differences between them. Serum of recipient No. 71, for instance, in contrast to the others, agglutinated the red cells of rabbit No. 292. The serum of recipient rabbit No. 177 differed from the others by agglutinating the red cells of rabbit No. 818. The serum of recipient No. 1236, in contrast to the others, agglutinated the red cells of rabbit No. 333. In a like manner it was possible to distinguish categories III and IV of rabbits.

Consequently the properties of the recipients' sera in relation to rabbits' red cells demonstrate the presence therein of common and different antigenic components. In spite of the similarity in the properties of the sera, we were unable to find any one pair of recipients whose sera would behave in the same way towards the red cells of a large number of rabbits; there were always greater or smaller differences between them. These findings demonstrate that the antigenic structure of the animals examined varies considerably. The presence in these rabbits of common antigenic components, responsible for the similarity in the properties of the red cells, is also confirmed by the results of the tests of the adsorptive properties of the red cells in relation to the immune sera of the recipients.

In Table 2 are given the results of the investigation of the serum of rabbit No. 1236 by means of the hemagglutination reaction before and after its adsorption with the red cells of 3 rabbits – Nos. 14, 866 and 756.

The red cells of rabbits Nos. 14 and 756, which gave a positive reaction with the serum of the recipient before adsorption, possessed the property of adsorption of antibodies from it. This can be seen from the fact that the serum of recipient No. 1236, after adsorption by red cells of rabbits Nos. 14 and 756, lost the power of ag-

glutinating rabbits' red cells. The red cells of rabbit No. 866, which reacted negatively with the test serum before adsorption, were incapable of adsorbing antibodies from it. The serum of rabbit No. 1236, after adsorption by the red cells of rabbit No. 866, did not lose its agglutinating properties in respect to the red cells of the donor (rabbit No. 1717) and of other rabbits (Nos. 14, 812, 756). The immune sera of other recipient rabbits behaved in precisely the same way after adsorption by the red cells of different rabbits. Thus rabbits, in spite of their considerable similarity antigenically, nevertheless show great variety. The antigenic differences between rabbits may be revealed during homografting by the formation of specific iso-antibodies in the recipient. The antigenic similarity of rabbits is presumably responsible for the agglutination of red cells of other rabbits as well as those of the donor by the immune serum of the recipient.

The results of the hemagglutination reaction and also of the adsorption of the sera by red cells show that the humoral changes arising in recipient rabbits during homoplastic skin grafting have the nature of specific immune iso antibodies. A study of the antigenic structure of rabbits, carried out by means of immune sera of recipients, showed the existence in rabbits of wide antigenic differences, which evidently lie at the basis of their biological incompatibility. Cross-testing of red cells and immune sera led to the segregation of the rabbits into several groups on the basis of the greater or lesser antigenic similarity between them.

SUMMARY

An investigation was carried out on immune sera obtained from 19 rabbit-recipients of skin homotransplants. The reaction of hemagglutination of these sera with erythrocytes of a large number (60) of rabbits was tested. On the grounds of the similarity of behavior of erythrocytes towards the immune sera, and of the immune sera with respect to the erythrocytes, the rabbits could be subdivided into several groups. However, there were antigenic differences between the rabbits even within a group. The antigenic similarities and differences of the rabbits were confirmed by the reaction of adsorption of the sera by the erythrocytes. The data obtained in these experiments make it possible to assume that the humoral changes produced as a result of skin transplantation are of the specific immune iso-antibody nature.

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THE USE OF THE METHOD OF ANAPHYLAXIS WITH DESENSITIZATION FOR THE STUDY OF THE ANTIGENIC PROPERTIES OF TUMORS DURING HETEROTRANSPLANTATION

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The method of anaphylaxis with desensitization is widely used in experimental and clinical oncology for studying the antigenic properties of normal and pathological tissues and, in particular, for analysis of the antigens of malignant tumors. The high sensitivity and specificity of the anaphylactic reactions enable the detection by this method of infinitesimal quantities of a protein when mixed with other proteins, and also allow a differential analysis to be carried out of the separate protein components entering into the composition of natural protein complexes.

In view of the special features of this method, we attempted to utilize it in the study of the antigenic properties of a tumor during heterotransplantation. We had in mind here to ascertain to what extent heterologous conditions of growth of the tumor would affect its antigenic properties.

EXPERIMENTAL METHOD

The test object was the mouse adenocarcinoma of Ehrlich. Carcinoma cells were transplanted into the cervical lymphatic nodes of a guinea pig. The lymphatic nodes, according to our previous investigation [1], are the most suitable organ for transplantation of the mouse tumor. As a rule the tumor shows growth in the lymphatic nodes in the course of 6-7 days, after which death of the carcinoma cells begins to take place on the 20th day, and the transplantate is completely absorbed.

In some experiments guinea pigs were sensitized to the original tumor, and in others to a 7-day heterotransplantate of this tumor. From 3-4 weeks from the moment of primary injection of protein, the guinea pigs were desensitized by intracardiac injection of mouse serum, of a saline extract of the heterotransplanted tumor and also of an extract of the original tumor. The order of injection of the antigens was changed according to the character of the experiment.

The use of the guinea pig as a recipient of the tumor and also for the reaction of anaphylaxis with desensitization appeared to have definite advantages for this particular investigation: it made it unnecessary to carry out desensitization of the animals to their own proteins (the proteins of lymphoid tissue), which are inevitably present in extracts prepared from heterotransplantates of the tumor.

EXPERIMENTAL RESULTS

In the first series of experiments (Table 1) guinea pigs were sensitized to mouse adenocarcinoma of Ehrlich. As seen from Table 1, guinea pigs sensitized to this antigen showed anaphylactic reactions to the injection both of mouse serum and of extract of the heterotransplantate of the mouse tumor. However, as the experiments showed, guinea pigs which were completely desensitized to extract of the heterotransplantate of the tumor did not lose the power to react to subsequent injection of the original antigen. The injection of an extract

TABLE 1

Records of the Experiments

Guinea pig no.	Sensitization	Desensitization										Assaulting injection	
	mouse adenocarcinoma	mouse serum						heterotransplantate of mouse				mouse adenocarcinoma	
	dose, mg	dose ml	reaction	dose ml	reaction	dose ml	reaction	dose mg	reaction	dose mg	reaction	dose mg	reaction
						Experiment							
390	15	0,5	Fatal shock	.	.	.	.	.	.	.	.	.	.
3965	15	0,5	» »	.	.	.	.	.	.	.		.	.
1375	15	0,3	++++	0,3	±	.	.	50	+	50	—	50	++++
2437	15	0,3	++++	0,3	±	.	.	50	+	50	—	50	Fatal shock
3128	15	0,3	++++	0,3	—	.	.	50	++	50	—	50	The same
2856	15	0,3	+++	0,3	±	.	.	42	+++	50	—	50	++++
2118	25	0,3	++++	0,5	++	0,5	—	50	++	50	—	50	Fatal shock
1896	25	0,3	++++	0,5	++	0,5	+	50	+++	50	±	50	++++
2539	25	0,3	+++	0,5	+	0,5	—	50	+++	50	—	50	Fatal shock
2508	25	0,3	++++	0,5	+	0,5	—	50	++++	50	±	50	The same
						Control							
2905		0,3	—	0,5	±	0,5	—	50	±	50	—	50	—
3674		0,3	—	0,5	—	0,5	—	50	—	50	—	50	—
3131		0,3	—	0,5	—	0,5	—	50	—	50	—	50	—

Conventional signs: — no reaction; +, ++, +++, ++++ reactions of different intensity; . no injection of serum given.

TABLE 2

Guinea pig no.	Sensitization: mouse adenocarcinoma + horse serum (washed 7 times)	Desensitization: horse serum	
	dose, mg	dose, ml	reaction
	Experiment		
5125	15	0.3	+
4214	15	0.3	++
3114	15	0.3	+++
	Control		
2741	.	0.3	–
1102	.	0.3	–

Conventional signs the same as in Table 1.

of the mouse tumor caused in all the guinea pigs a severe anaphylactic shock, terminating fatally in about half the animals included in the experiment. Control, nonsensitized guinea pigs, injected along with the animals of the experimental group with the same doses of the antigens mentioned, in no case developed signs of anaphylactic shock.

The results given demonstrate that the antigens of the original tumor are preserved in the heterotransplantate. The reaction of the guinea pigs to injection of an extract of the heterotransplanted tumor was shown to be present after they had been completely desensitized to the species antigen (mouse serum); for this reason it may be asserted that the antigenic similarity between the tumor before and after heterotransplantation is determined by a complex of antigens, which differs in its properties from the species antigen of the tumor.

Meanwhile the experiments also show that in addition to common antigenic properties, certain antigenic differences are also present between the tumor before and after heterotransplantation. This is demonstrated by the fact that the extract of heterotransplanted tumor did not entirely remove the sensitivity of the guinea pigs to the subsequent injection of an extract of the original tumor. For an explanation of this phenomenon we postulated that the difference in the antigenic properties of the tumors being compared is determined not by the loss of certain antigens of the tumor at the time of heterotransplantation, as might be thought, but because this difference is connected with a change in the antigenic properties of the tumor under the influence of heterogenic protein.

In fact the extract of the heterotransplanted tissue used in the experiments as an antigen for desensitization of the guinea pigs, besides the proteins of the carcinoma cell, naturally contained a vast quantity of the proteins of lymphoid tissue. It is very probable that under such conditions the heterogenic proteins, firmly adsorbed onto the surface of the tumor cells, could to some extent block the active groups of tumor antigens and thereby restrict the antigenic powers of these antigens in relation to the appropriate receptors of the tissues of the sensitized animal. We attempted to check the grounds for this hypothesis experimentally. For this purpose we carried out a special experiment in which guinea pigs were sensitized to cells of ascitic carcinoma which had, as a preliminary measure, been placed in contact for 16 hours with horse serum at a temperature of 37°C, and then washed free from serum seven times with physiological saline.

The experiments showed (Table 2) that contact of the carcinoma cell with heterogenic serum leads to the firm adsorption of the heterogenic protein on the surface of the carcinoma cells, which can only be removed with difficulty by extensive washing of the carcinoma cells in physiological saline.

The effect of heterogenic protein on the antigenic activity of the tumor extract was examined by G. V. Suvorova [2], who succeeded in demonstrating by means of the complement fixation reaction that the intensity of the reaction between the antigens of the tumor and the corresponding immune sera is reduced if the tumor from which the antigen was prepared was placed as a preliminary measure in contact with heterogenic serum for a few hours. The weakening of the reactivity of the carcinoma antigen, in the author's opinion, is connected with the adsorption of foreign protein onto the surface of the tumor protein.

In another series of experiments to study the antigenic properties of a heterotransplanted tumor we attempted to shed light on the fate of the species antigen of the tumor after heterotransplantation. In contrast to the previous experiments, the guinea pigs were sensitized not to the original tumor but to a heterotransplantate of this tumor in the lymphatic nodes of the guinea pig.

The results of these experiments are shown in Table 3.

As seen from the findings in Table 3, guinea pigs sensitized to a heterotransplantate of the tumor were insensitive to the mouse serum. On subsequent injection of an extract of mouse tumor the guinea pigs reacted by anaphylactic shock. It was further discovered that desensitization to the mouse tumor caused total loss of

TABLE 3

Guinea pig no.	Sensitization	Desensitization												Assaulting injection	
	heterotransplantate of mouse adenocarcinoma	mouse serum				mouse adenocarcinoma								heterotransplantate of mouse adenocarcinoma	
	dose (mg)	dose (ml)	reaction	dose (ml)	reaction	dose (mg)	reaction	dose (mg)	reaction	dose (mg)	reaction	dose (mg)	reaction	dose (mg)	reaction
						Experiment									
958	25	0,4	—	0,3	—	60	++++	60	++++	60	++++	60	+	50	—
386	25	0,5	—	0,3	—	60	++	60	+++	60	+(+)	60	—	50	—
1604	25	0,5	±	0,3	—	60	+++	60	++++	60	++(+)	60	—	50	—
						Control (horse serum)									
1396	25	0,4	—	0,3	—	60	++++	50	+++	60	++	60	+	50	—
1658	25	0,4	+	0,3	—	60	++++	50	+	60	+++	60	—	50	—
555	25	0,3	+	0,3	—	60	++	50	++	60	+++	60	—	50	—

TABLE 4

Guinea pig no.	Sensitization: heterotransplantate of mouse adenocarcinoma	Desensitization: mouse serum								mouse adenocarcinoma	
	dose (mg)	dose (ml)	reaction	dose (ml)	reaction	dose (ml)	reaction	dose (ml)	reaction	dose (mg)	reaction
					Experiment						
2054	15	0,3	++	0,4	++	0,5	+	0,5	—	50	Fatal shock
3194	15	0,4	+++	0,4	++	0,4	+	0,5	—	30	The same
3043	15	0,3	++	0,4	—	0,4	+(+)	0,5	±	20	» »
2604	15	0,3	++	0,4	±	0,4	+(+)	0,5	—	20	» »
					Control						
2813	.	0,3	—	0,4	—	0,5	±	0,5	—	50	—
2402	.	0,3	—	0,4	—	0,5	—	0,5	—	50	--
3930	.	0,3	—	0,4	—	0,5	—	0,5	—	50	—

the sensitivity of these animals to the original antigen, i. e. to an extract of the heterotransplanted tumor.

In Table 4 are shown the results of a similar experiment. In contrast to the previous experiment guinea pigs were sensitized to a heterotransplanted tumor showing intensive growth in the lymphatic nodes of a guinea pig. This experiment showed that in this particular case sensitization of the guinea pigs was developed both to mouse serum and to the tumor. In these circumstances there was observed a more or less moderate reaction to the species protein and a violent reaction to the protein of the malignant tumor, terminating in the death of all the experimental animals. In some guinea pigs death ensued after the injection of a comparatively small dose of the carcinoma antigen. Unsensitized animals did not react to the injection of these antigens mentioned.

The results in Tables 3 and 4 demonstrate that a heterotransplantate of a tumor has in its composition antigens which are identical with the antigens of the mouse tumor. In addition, by means of mouse serum, carrying protein with species-specific properties, it is possible to show that a species antigen forms an indispensable component of the antigenic complex of the carcinoma cell. Analysis of the results of these experiments shows that the species antigen in the tumor heterotransplantate stands in a definite relationship with the remaining antigenic components of the carcinoma cell. If, for instance, in the first case the reaction of the guinea pigs to mouse serum is absent and the reaction to the tumor is moderate, then in the second case a clear reaction will be observed to the species antigen and a violent reaction of shock, terminating in the death of all the animals taking part in the experiment will occur in response to the injection of the remainder of the antigenic complex of the carcinoma cell.

Furthermore guinea pigs sensitized to a heterotransplanted tumor, in contrast to guinea pigs sensitized to unchanged tumor, are desensitized to the corresponding antigen with great difficulty. As a rule frequent injections of antigen are required in order to obtain complete desensitization of the animal.

As the experiments showed, the sensitivity to protein of the carcinoma cell (see Table 3) was removed only after the guinea pigs had received 4 injections of comparatively large doses of antigen. Complete desensitization to the species antigen (see Table 4) also took place after 4 injections of mouse serum. Meanwhile complete loss of sensitivity of the guinea pigs sensitized to the nontransplanted tumor is attained as a rule after two injections of a suitable dose of serum.

It can be concluded on the basis of the results obtained that the method of anaphylaxis with desensitization in guinea pigs enables a highly accurate analysis to be made of the antigenic properties of heterotransplanted tumors. By means of this method it was possible to show that the temporary growth of a mouse tumor in an animal of another species does not lead to a sharp change in its antigenic properties. Under these conditions the heterotransplantate of the tumor largely preserves the antigens of the original tumor and, in particular, those antigens which characterize the species to which the proteins of the tumor cell belong.

Certain antigenic differences found in the experiments between the original and the heterotransplanted tumors are connected, in our opinion, with physicochemical changes in the reactive properties of the tumor proteins as a result of the adsorption of foreign protein. At the same time, during investigation of the antigenic properties of tumors after long-standing heterotransplantation, it must be considered that the changes of this sort in the tumor are associated not only with adsorption of proteins but also with the loss of some of the tumor antigens in the process of heterotransplantation.

For a final solution of these problems additional investigations will be required in order to minimize the opportunity of a dual interpretation of this phenomenon.

SUMMARY

The method of anaphylaxis with desensitization makes it possible to analyze the antigenic properties of the heterotransplanted tumors in guinea pigs with considerable precision. It was demonstrated that the temporary growth of the mouse tumor in an animal of another species does not alter considerably its antigenic properties. The antigens of the original tumor, particularly the antigen characterizing the species specificity of the protein, are preserved in the heterotransplant of the tumor in these conditions.

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DETERMINATION OF THE SEROLOGICAL ACTIVITY OF THE DESOXYRIBONUCLEOPROTEINS (DNP) OF HUMAN GASTRIC CARCINOMA TISSUES AFTER ADSORPTION ON COTTON FABRIC

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V. S. Gostev and D. G. Grigor'ian [1, 2, 3] devised a new method of serological study of the desoxyribonucleoproteins (DNP), based on the specific fixation of the proteins of antisera on cotton fabric adsorbents soaked with DNP.

In the present investigation our aim was to ascertain the possibility of studying the serological activity of DNP in another reaction – in the quantitative complement fixation reaction.

The CFR method of Heidelberger [6] and of A. P. Konikov [4] was used by V. S. Gostev and N. A. Shagunova [2, 5] with a 50% titer, and modified to study the azoproteins fixed on paper. We utilized this method for investigating the serological activity of desoxyribonucleoproteins adsorbed on cotton fabric.

Preparation of test antigens of DNP. Nuclear DNP were obtained from material removed at operation – tissue of human gastric carcinoma – by the method described in the paper by V. S. Gostev and D. G. Grigor'ian [1].

Preparation of antisera. Sera were obtained by the intraperitoneal immunization of rabbits with suspensions of both human gastric carcinoma tissues and human thyroid gland. For immunization of the animals only tissues removed at operation were used. It was shown in our laboratory [6] that the immunization of animals with postmortem material does not lead to the formation of antibodies to crude DNP on account of the fact that DNP is present in postmortem tissue in a state of destruction. The animals were injected on alternate days with increasing doses of protein which were estimated as nitrogen. Exsanguination of the rabbits was carried out on the 8th and 12th days after the last immunization, from the auricular vein. The sera were heated for 30 minutes at 56°C and stored at 2-4°C. Boric acid was used as a preservative.

Quantitative CFR at a 50% titer with adsorbents impregnated with DNP from human gastric carcinoma tissue. The protein nitrogen of the antigen was estimated by Conway's method, and a red cell suspension for the hemolytic system was standardized on the electrophotocolorimeter. The complement was titrated as a first stage and for the reaction an excessive number of units of complement was always taken in order to ensure that it was not all entirely fixed in either the control or the experimental tests. The complement which remained unfixed was titrated, and by the difference in the titers of the control and experimental reactions, the degree of fixation in units of complement was established. A unit of complement was taken to be that quantity of it (in a volume of 0.5 ml) which, after keeping in an incubator at 37°C, brings about 50% hemolysis of red cells in 0.5 ml of hemolytic system.

The present reaction, like the classical form, is composed of two stages. The first stage – fixation of complement by a specific antigen – antibody complex – takes place in the cold. A cotton fabric test antigen (see preparation of test antigen) was placed in a tube in 0.3 ml of physiological saline, and to this was added

Quantitative CFR at a Titer of 50% with DNP from Human Gastric Carcinoma Tissue, Adsorbed on Cotton Fabric

	Antigen	Antiserum to human gastric carcinoma (slurry of surgical tissue)		Antiserum to human thyroid gland (slurry of surgical tissue)		Serum of an unimmunized rabbit		Control of antigen	
	Units of complement	free	fixed	free	fixed	free	fixed	free	fixed
Experiment	DNP from human gastric carcinoma	–	31	12.8	18.2	31	–	31	–
Control	Clean cotton fabric	31	–	31	–	31	–	31	–
Control	Control of serum	31	–	31	–	31	–	31	–
Control	Control of complement	31	–	–	–	–	–	–	–

0.2 ml of antiserum (dilution 1:10) and 0.1 ml of complement, either whole or diluted, depending on the strength of the complement. The volume of the reacting mixture in the first stage of the reaction was 0.6 ml. The experiment was accompanied by suitable controls of the antigens, the antiserum, and the complement.

The second stage of the reaction consisted of back-titration of the complement remaining unfixed. For this purpose, on another day from each experimental and control tube definite doses of the reacting mixture (from 0.01 to 0.1 ml) were made up to a volume of 0.5 ml with physiological saline, and an equal volume of hemolytic system was added. The tubes were incubated at 37°C for 30 minutes. Later, after cooling and centrifugation of the tubes the coefficient of extinction of the supernatant fluid was determined by the electrophotocolorimeter. The percentage of hemolysis was determined from the calibration curve expressing the relationship between the percentage hemolysis and the coefficient of extinction.

In order to establish the content of unfixed complement in units in the sample under examination, doses of the reacting mixture were chosen so that the percentage of hemolysis lay between limits of 20 and 80. It was possible only comparatively rarely to determine the dose of complement giving 50% hemolysis. In such cases 50% hemolysis was determined according to the dose of complement causing partial hemolysis by means of Krogh's formula:

$$\lg x_2 = \lg x_1 - n \lg \frac{y}{100-y},$$

where: x_2 is the amount of complement causing 50% hemolysis; x_1 the amount of complement taken for the experiment; n the coefficient under these particular experimental conditions, 0.2; and y is the percentage hemolysis observed in the experiment.

Starting from a titer of 50%, the number of units of complement present in the test sample was calculated.

In order to obtain the value of the specific fixation it is necessary to subtract the number of units of free complement in the experiment not from the total number of units of complement taken in the experiment, but from the number of free units in the control if this is smaller than the total number on account of the presence of anticomplement. For this subtraction, the control is taken in which the anticomplementary properties are more strongly expressed. Where the anticomplement content of the antigen or antiserum is high, it is advisable to increase the dose of complement slightly, although very large doses reduce the accuracy of the reaction.

Figures from a typical record of an experiment, obtained during a serological study of the DNP adsorbed on cotton fabric, using the method as described above, are given in the Table.

EXPERIMENTAL RESULTS

As seen from the Table, the DNP of human gastric carcinoma tissue shows a high serological activity, specifically fixing complement in the presence of antiserum. DNP from human gastric carcinona tissue, when combined with antiserum to human thyroid gland, fixes only 18.2 units of complement out of 31, whereas the same DNP with antiserum to human gastric carcinoma tissue fixes the entire stock of free units of complement taken in the experiment.

Thus DNP, fixed on cotton fabric adsorbents, are serologically active and may be studied by means of the quantitative complement fixation reaction at a titer of 50%.

SUMMARY

The author investigated the quantitative complement fixation test with desoxyribonucleoproteins adsorbed on cotton fabric. The serological activity of desoxyribonucleoproteins and the possibility of their examination by this method was demonstrated.

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ANTIGENIC DIFFERENTIATION OF THE GREY AND WHITE MATTER OF THE BRAIN IN MAN

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It is known that the grey and white matter of the cerebral hemispheres differ from each other not only morphologically but also in their biochemical composition. The grey matter contains more water-soluble protein and less insoluble protein residue than the white matter [10]. The grey matter also differs from the white in its lower lipid content, and on the other hand, in the greater activity of its enzymes responsible for carbohydrate metabolism (adenosine triphosphatase, aldolase) and in other properties.

The differences in the structure of the grey and white matter of the brain and also in their biochemical composition and function gave us grounds for the belief that these two forms of tissue are characterized by qualitative antigenic differences also. However, this question has not yet been answered sufficiently convincingly in view of great difficulties in the devising of methods for reaching a solution.

Reichner and Witebsky [12] immunized rabbits with the grey and white matter of the brain of cattle, and obtained sera which reacted more intensively in the complement fixation reaction with antigens from the same brain tissue as was used for the immunization. These two forms of serum also had other differences. The sera to the white matter reacted with antigens from the brain of fishes but did not react with antigens from the brains of mammalian embryos – man and guinea pig. Conversely sera to the grey matter reacted with antigens from the embryonic brains but did not react to the brains of the fishes. On the basis of these findings these workers came to the conclusion that antigenic differences existed between the grey and white matter of the brain.

Different results were obtained by A. F. Makarchenko [9], who was unable to establish, by means of the complement fixation reaction, any antigenic differences between the grey and white matter of the brain in dogs. V. A. Korenevskaia reported that by the use of L. A. Zil'ber's method of anaphylaxis with desensitization it is possible to detect antigenic differences between the grey and white matter of the brain in man [1].

The method of selective absorption developed in our own laboratory [2-8], enabling the demonstration of specific antigens in normal and pathological tissues, was used by us in a study of the differentiation of the antigenic properties of the grey and white matter of the brain in man.

EXPERIMENTAL METHOD

Antigens from the grey matter were obtained from thin sections of the surface of the cerebral hemispheres, and antigens from the white matter – from areas of tissue from the cerebral peduncles. One g of tissue was ground in a porcelain mortar, 10 ml of a 0.85% solution of sodium chloride being gradually added. The homogeneous emulsion was centrifuged at 2000 rpm for 15 minutes; the supernatant fluid was titrated for its content of anticomplement in order to select the working dose of antigen, and it was then tested with the brain antiserum in the complement fixation reaction at 37°C.

In order to study the antigenic properties of the grey and white matter of the brain, in the first place we

TABLE 1

Immunological Activity of Brain Antisera with Antigens from the Grey and White Matter of the Human Brain

Serial no. of serum	Dilution	Antigens from tissue		
		of the grey matter	of the white matter	of the liver
79	1 : 20	++++	±	±
	1 : 40	++++	—	—
	1 : 80	++++	—	—
	1 : 160	+++	—	—
85	1 : 30	++++	—	—
	1 : 60	++(+)	—	—
	1 : 120	++	—	—
	1 : 240	+	—	—
116	1 : 20	++++	±	—
	1 : 40	++++	—	—
	1 : 80	+++	—	—
	1 : 160	++	—	—
120	1 : 30	+++	—	—
	1 : 60	+++	—	—
	1 : 120	++	—	—
	1 : 240	+	—	—

used sera of horses, immunized with an emulsion of the brains of mice inoculated with the viruses of tick-borne and Japanese encephalitis [11]. These sera, as shown by our preliminary investigations, contain, besides antibodies to the viruses of these forms of encephalitis, antibodies to antigens from the brain of various species of mammals, including human brain [8]. The specificity of the sera was checked by testing them simultaneously with antigens from liver tissue prepared by the same method as the antigens from brain tissue.

EXPERIMENTAL RESULTS

The results of the investigation of the immunological activity of the brain antisera obtained from horses with antigens from the grey and white matter of human brain are shown in Table 1.

As seen from Table 1, the sera of series 79, 85, 116 and 120 gave a positive complement fixation reaction with antigens obtained from the grey matter of human brain, and at the same time did not react with antigens from the white matter. An indication of the specificity of these sera was their failure to fix complement with antigens from human liver tissue. These experiments thus showed that the sera of horses immunized with mouse brain tissue are capable of differentiating the antigenic properties of the grey matter of the human brain from antigens of the white matter.

Further confirmation of this was given by experiments which we carried out with the sera of rabbits immunized with the white matter of human brain. We used the white matter in view of its greater homogeneity, for it is difficult to obtain grey matter uncontaminated with white.

Rabbits were injected intraperitoneally with a 10% emulsion of white matter in doses of 2 ml on 4 occasions at intervals of 4 days between the injections. From 8-10 days after the last injection the serum was obtained and tested against saline extracts of the white and grey matter of human brain. When the experiment was performed in this way it was impossible to observe any great difference in the reaction of the sera with antigens from the white and grey matter. However, we clearly observed differences here in a specific absorption reaction. For this purpose we used fresh crude white and grey matter tissue from human brain. Before absorption, the tissues were repeatedly rinsed with physiological saline until the supernatant fluid was completely translucent. To one volume of precipitate of washed tissue was added 4 volumes of serum in a dilution of 1:10. The mixture of serum and tissue was kept in a refrigerator at +4°C for 18 hours, after which it was centrifuged at 2000 rpm for 30 minutes; the supernatant fluid was again centrifuged at 8000-10,000 rpm for 30 minutes and then tested in the complement fixation reaction with extracts of the white and grey matter of the brain and also of liver (specificity control of the serum).

The results of these investigations are shown in Table 2, which is actually one of the records from a large number of experiments of the same type.

It is seen from Table 2 that rabbit antiserum obtained to white matter of the human brain also reacted, before absorption, with antigens from the grey matter of the brain, but did not react with extracts from the liver, i. e. in these particular dilutions it showed specific activity toward the brain antigens. The quantitative complement fixation reaction showed only a slightly higher value with antigens from the white matter of the brain as compared with antigens from the grey matter. After absorption with grey matter this serum completely lost its power to react with an extract of grey matter and at the same time retained its activity toward the antigen

TABLE 2

Comparative Study of the Antigenic Properties of the Grey and White Matter of the Human Brain by the Absorption Method

Sera	Dilution	Antigens from tissue		
		of the white matter	of the grey matter	of the liver
Immune rabbit serum to the white matter of human brain before absorption	1 : 100	++++	++++	—
	1 : 200	+++ (+)	+++	—
	1 : 400	+++	++ (+)	—
	1 : 800	++	+ (+)	—
Same serum after absorption with grey matter of human brain	1 : 20	+++	—	—
	1 : 40	++	—	—
	1 : 80	+	—	—
	1 : 160	±	—	—
Same serum after absorption with white matter of human brain	1 : 20	—	—	—
	1 : 40	—	—	—
	1 : 80	—	—	—
	1 : 160	—	—	—
Horse serum obtained by immunization with mouse brain tissue	1 : 20	±	++++	—
	1 : 40	—	+++	—
	1 : 80	—	++ (+)	—
	1 : 160	—	++	—

from the white matter of the brain. Conversely, as a result of treatment of the serum with white matter of the brain to which the serum had been produced, complete absorption of antibodies to antigens from white and grey matter took place.

The power of selective absorption of homologous antibodies from a serum, which is inherent in the white matter of the brain, demonstrates that antigens are present in this tissue which are absent from the grey matter.

An extract of white matter in the same experiment (see Table 2) did not react with a horse serum containing antibodies to grey matter alone.

Our investigations thus showed that the grey and white matter of the human brain have different antigenic constitutions. The grey matter contains antigens not present in the white matter, and vice versa.

The grey matter of the cerebral hemispheres in man contains antigens which are similar to the brain antigens of certain animals (mice), but these antigens are absent from the white matter of the human brain.

A comparative study of the antigenic structure of the brain in man and animals is of value towards understanding the mechanism of encephalitic phenomena sometimes observed as a result of the use of preparations from the brains of animals for purposes of treatment or prophylaxis.

SUMMARY

Saline extracts of the grey and white matter of the human brain were tested by the complement fixation reaction with various anticerebral sera.

Rabbit serum obtained after immunization by white matter of the human brain enabled precise differentiation of the antigen of the white matter from that of the grey matter after the absorption by the tissue of the grey matter. The difference in the immunological properties of the grey and white matter of the human brain was also revealed in investigation of the horse sera of horses immunized with a cerebral emulsion of mice. There was a selective reaction of these sera with the antigens of the grey matter (as compared with the antigens of the white matter). Thus, the results which were obtained show the presence of an antigenic difference between the tissues of the grey and white matter of the human brain.

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ONCOLOGY

DISTINCTIVE FEATURES OF THE DEVELOPMENT OF M-1 TUMORS IN RATS DURING CHANGES IN THE FUNCTIONAL CONDITION OF THE NERVOUS SYSTEM

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Recently pathological physiologists and clinicians have been paying more and more attention to the importance of the functional condition of the central nervous system in the pathogenesis of tumors.

In this connection there is particular interest in the research into the role of functional trauma of the central nervous system in the development of tumors. In our laboratory, P. Ia. Novorasova [6] showed that preliminary, prolonged trauma to the higher divisions of the central nervous system in mice by means of an electric current accelerated the development of the ascitic form of adenocarcinoma. Conversely trauma of brief duration to mice by an electric current retarded growth of the tumors. A. N. Kursheva [3], in experiments on rats, also noted that growth of an inoculated M-1 tumor was dependent on the original condition of the central nervous system. In these investigations attention was drawn to the importance of the type of nervous activity. S. I. Lebedinskaia and A. A. Solov'ev [5] also demonstrated the importance of the type of nervous behavior of the animals. They found that in rats with a strong type of nervous system a tumor developed more slowly than in rats with a weak type, in which the weight of the tumor reached considerable magnitudes; a neurotic state of the rats intensified tumor growth. Similar results were obtained by F. M. Khaletskaia [11] in experiments on mice by overstraining the activity of the nervous system, and by E. I. Vodakova and O. A. Serdiukova [1] in experiments on rats of various ages with trauma to the nervous system.

The present investigation is devoted to the study of the growth of tumors in rats with different types of nervous behavior after injection of small and large doses of caffeine.

It was shown by the observations of I. P. Pavlov and his co-workers that caffeine increases the excitation of the cortical cells [2, 4 and others]. Later investigations showed that the injection of small doses of caffeine improves differentiation but large doses bring about limiting inhibition [8, 9, 10].

EXPERIMENTAL METHOD

As a preliminary measure a system of conditioned reflexes was produced over a period of 4 months in 28 experimental rats by the motor-alimentary method of Kotliarevskii. After the establishment of a dynamic stereotype in the rats, in order to define more precisely the type of nervous activity, experiments were carried out in which the stereotype was changed (the rate of extinction and restoration of the conditioned reflexes was determined) and the caffeine test was applied. As a result, the type of nervous activity of the rats was subdivided into strong excited, strong inert and weak.

Of the 28 experimental animals 16 were included in the group of experiments to study the effect of small doses of caffeine on the development of M-1 tumors.

In order to study the effect of large doses of caffeine on the growth of tumors the other 12 rats were used. It was established that to increase the process of excitation in the nervous system of the rats, the small doses of caffeine used must differ according to the type of nervous behavior of the animal: for rats of a strong

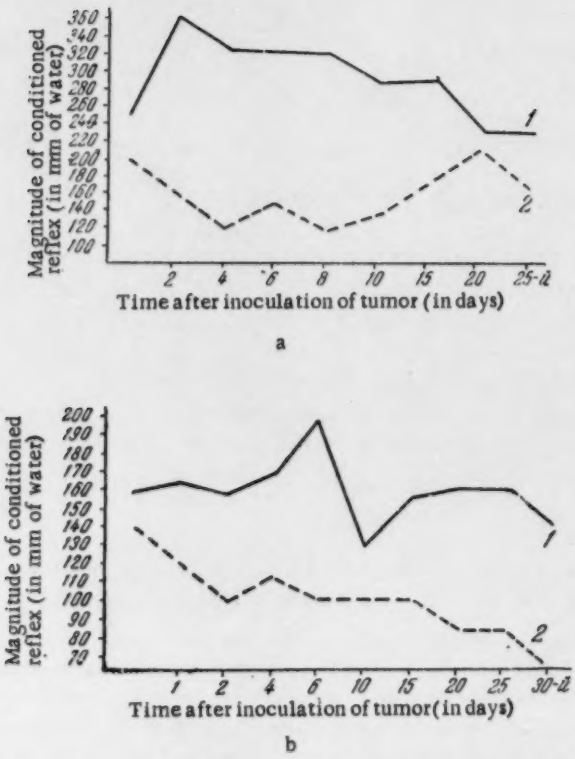

Fig. 1. Curves showing the changes in the total magnitude of the conditioned reflexes in rats of strong (1) and weak (2) types of nervous activity, receiving small doses of caffeine, after inoculation of a tumor (a); control experiments (b).

and balanced type the dose should be 0.002 g, and for rats of a strong excited and weak type, 0.001 g (body-weight 200-220 g). Using different doses of caffeine in all nine experimental rats (7 with a strong and 2 with a weak type of nervous system), we noted improvement of differential inhibition and high functional mobility of the nervous processes; 7 animals (5 with strong and 2 with weak types of nervous system) acted as controls (inoculation of the tumor without injection of caffeine).

The large doses of caffeine which were used also varied according to the type of nervous behavior of the animals: for rats of a strong balanced and strong excited type the dose was 0.05 g, and for rats of weak type – 0.01 g (body weight of the rats 200-220 g). During the injection of large doses of caffeine 2 rats with a strong inert type of nervous system died. In 3 rats of strong excited type and 2 rats of weak type of nervous system, severe disorders of conditioned reflex activity were observed. In this group 5 rats acted as controls (3 with a strong excited type and 2 a weak type of nervous system). Caffeine was injected subcutaneously in all the rats for a period of 10 days before inoculation of the tumor, and daily for 25 days after inoculation. Inoculation of the tumor in the first and second groups was carried out from different rats affected by tumors.

EXPERIMENTAL RESULTS

In rats of strong excited and strong inert types of nervous system, receiving small doses of caffeine, the tumor developed more slowly and did not reach a large size; in rats with a weak type of nervous activity the tumor developed more rapidly and attained a large size.

In animals with a strong type – both excited and inert – of nervous system, receiving small doses of caffeine, development of the tumor was observed on the 7th-9th day after transplantation; in the control group the tumor was already apparent on the 3rd-4th day. The average weight of the tumor in the rats with a strong

type of nervous system on the 25th day was 9 g, and in the control rats 17.2 g; in the rats of weak type of nervous activity it was 42 g.

On comparing the character of the changes in the total magnitude of the conditioned reflexes (the sums of the magnitudes of the motor reflexes of the rat throughout one session) in a rat with a strong type of nervous activity taking part in the experiment (Fig. 1, a) and in a control rat (Fig. 1, b) we observed that caffeine brought about an increase in the strength of the conditioned reflexes and maintained the strength of the excitatory processes, and it was this latter factor which restrained the development of the inoculated tumor. In rats with a weak type of nervous system, on both experimental (see Fig. 1, a) and control (see Fig. 1, b) groups the transplanted tumor was found to be too powerful a stimulus; this led to the development of limiting inhibition in the nervous system, to a fall in the magnitude of the conditioned reflex and, consequently, to a weakening of the defense mechanisms and to acceleration of the growth of the tumor.

Confirmation of this point of view is obtained from experiments in which large doses of caffeine were used. In all the rats receiving large doses of caffeine there were severe disorders of conditioned reflex activity. A weakening and dying away of the positive conditioned reflexes, together with disinhibition of differential inhibition were observed, which may account for the presence of phasic states in these animals.

Observation of the development of the tumor in rats receiving large doses of caffeine showed that the tumor appeared in these animals on the 3rd-4th day after inoculation, and it reached a large size; after 25 days the weight of the tumor varied between 67 and 100 g. In the control rats of this group the weight of the tumor averaged 49 g.

On the basis of the results obtained it may thus be postulated that small doses of caffeine, which normalize the functional condition of the nervous system in rats during the growth of a tumor, facilitate the appearance of protective mechanisms in the process of development of an inoculated tumor; large doses of caffeine lead to disturbance of the functional state of the nervous system and to the development of limiting inhibition.

Our findings are in agreement with those obtained by S. P. Sizenko [7] in experiments in which small doses of caffeine and bromide were used during x-ray therapy of experimental sarcomas in rats. In these experimental conditions this author observed an enhanced therapeutic effect.

SUMMARY

It was shown in experiments on rats that small doses of caffeine inhibit the development of the transplanted tumor (sarcoma M-1) by increasing the functional mobility of the nervous system. Large doses of caffeine accelerate the growth of the tumor in connection with the development of the limiting inhibition in the central nervous system.

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CHANGES IN THE PROPERTIES OF THE SERUM DURING GROWTH OF A BROWN-PEARCE CARCINOMA IN RABBITS

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In recent years the problem of the immunology of cancer has received the most extensive study. The body responds to the development of a tumor by definite immunological reactions [1, 2, 4, 7, 9]. Besides the natural immunity to the development of malignant tumors [8], some research workers [5] distinguish 2 further forms of immunity: immunity to an already developed tumor and antimetastatic immunity.

Certain authors [3, 8] have shown that in persons with malignant tumors, diagnosed at early stages, in some cases it is possible to discover specific anticarcinoma antibodies. Moreover at later stages of the malignant disease these antibodies can no longer be found [8].

It has been shown [11] that feeble metastasis of a tumor is associated with early appearance of specific anticarcinoma antibodies. In animals with well marked metastases, specific anticarcinoma antibodies appear very late or may not be found at all.

It may be thought from these and other findings that a tumor, developing in the body, may exert a specific effect on the body by suppressing its power of antineoplastic immunological reaction. The present paper is devoted to the study of this problem.

EXPERIMENTAL METHOD

As experimental material we selected the strongly metastasizing Brown-Pearce rabbit adenocarcinoma. All the experiments were carried out with tumors of the same strain. Experiments were performed on 2 groups of sexually mature male rabbits of the chinchilla breed, weighing 2.5-3 kg; there were 11 animals in each group. The tumor was inoculated intratesticularly in the rabbits of both groups. Before inoculation of the tumor and then every 3rd day afterwards, 10 cc of blood was taken from each rabbit, and serum was prepared from this

TABLE 1

Results of Morbid Anatomical Examination of Rabbits After Inoculation with Brown-Pearce Adenocarcinoma

Time of examination of rabbits after inoculation, in days	Total no. of rabbits dying and killed	Tumor without metastases	Tumor with metastases
From the 6th to the 10th	5	2	3
" " 10 " " 16	13	1	12
" " 16 " " 25	6	–	6

TABLE 2

The Course of the Complement Fixation Reaction of Serum No. 740 (Antigens: from a Brown-Pearce Tumor, from Rabbit's Testicle, from Rabbit's Liver)

Time of taking blood	Antigens	Dilution of sera		
		1:10	1:20	1:40
Before inoculation of the tumor	Brown-Pearce tumor	+++	++	+
	Testicle	+++	++	+
	Liver	+++	++	+
On the 3rd day after inoculation of the tumor	Brown-Pearce tumor	+++	++	+
	Testicle	+++	++	+
	Liver	++++	++(+)	++
On the 6th day after inoculation of the tumor	Brown-Pearce tumor	++++	++(+)	+
	Testicle	++++	++(+)	+
	Liver	++++	+++(+)	++
On the 9th day after inoculation of the tumor	Brown-Pearce tumor	+++	++	+
	Testicle	+++	++	+
	Liver	++++	+++	+
On the 12th day after inoculation of the tumor	Brown-Pearce tumor	++	+(+)	±
	Testicle	++	+(+)	±
	Liver	+++	++	+
On the 15th day after inoculation of the tumor	Brown-Pearce tumor	+	+	±
	Testicle	+	+	±
	Liver	++	++	+
On the 18th day after inoculation of the tumor	Brown-Pearce tumor	+	±	h
	Testicle	+	±	h
	Liver	++	+	+
On the 21st day after inoculation of the tumor	Brown-Pearce tumor	+	±	h
	Testicle	+	±	h
	Liver	++	+	±
On the 24th day after inoculation of the tumor	Brown-Pearce tumor	+	±	h
	Testicle	+	±	h
	Liver	++	+	+
On the 29th day after inoculation of the tumor	Brown-Pearce tumor	±	h	h
	Testicle	±	h	h
	Liver	+	±	h

Note: The letter h signifies complete hemolysis.

blood. After each removal of blood, the animals were injected subcutaneously with physiological saline, also in a volume of 10 cc. All the sera obtained were inactivated at 56°C and tested for anticomplement.

In order to determine the presence of antibodies in the sera obtained, and to assess their specificity, we utilized the complement fixation reaction. Fixation of complement took place in the cold (at a temperature of 3-5°C). As antigens in the reaction we used saline extracts of a Brown-Pearce tumor, and also of tissue from the liver and testicles of both healthy rabbits and of rabbits affected by tumors.

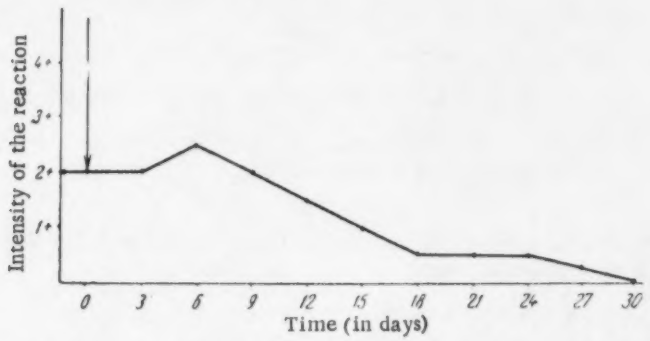
In each experiment parallel tests were made of sera taken from the same animal before inoculation with the tumor and at different periods after inoculation. Simultaneously, on the basis of pathological anatomical investigation, in a third group of rabbits the time of appearance of metastases was determined in animals which had been inoculated with a tumor. In this group observations were made altogether on 24 rabbits.

EXPERIMENTAL RESULTS

The results of the examinations are shown in Table 1. As seen from the findings given in this table, on the 10th-16th day after inoculation metastases, visible to the naked eye, were found in 18 rabbits of the third experimental group; on the 26th day after inoculation metastases were found in all the animals without exception. The results in Table 1 thus show that metastases in animals affected by tumors are first found starting on the 10th day. Sixteen days after inoculation metastases were present in all the rabbits examined.

The results of the experiments to study the sera of rabbits already affected by a Brown-Pearce tumor are given in Table 2. As may be seen from Table 2, which is a typical record of an experiment, the sera of the rabbits taken before inoculation of the tumor reacted positively at the same titer and with the same intensity both with antigen from the Brown-Pearce tumor and with antigens from normal tissue of the testicle and liver of a rabbit. The same may be said of the sera obtained from the rabbits on the 3rd-9th day after inoculation.

All these sera reacted with the above named antigens +++ or ++++ in a dilution of 1:10, ++ or +++ in a dilution of 1:20, and + in a dilution of 1:40. The sera obtained from rabbits on the 12th and 15th day after inoculation of the tumor gave a positive reaction of ++ or +++ in a dilution of 1:10, + or ++ in a dilution of 1:20, and in a dilution of 1:40 a positive reaction of + was observed only with antigen from normal liver tissue. The sera obtained on the 18th-29th day after inoculation gave a feebly positive reaction in a dilution of 1:10 only. In higher dilutions the reaction was to all intents and purposes negative.



Changes in the titer of normal antibodies (serum No. 740 in a dilution of 1:20) in the course of the experiment. Inoculation of the Brown-Pearce tumor is indicated by the arrow.

Analysis of these findings showed that specific antibodies cannot be found in the sera of rabbits already affected by Brown-Pearce tumors. It further follows from these results that the sera of these rabbits evidently contain normal antibodies to tissues from rabbit's organs. The titer of these antibodies begins to fall on the 12th day after inoculation, and on the 29th day they are practically absent. The changes in the titer of the normal antibodies during the course of the experiment are shown graphically on the figure.

It must also be pointed out that all the sera reacted with slightly greater intensity with antigen from normal liver tissue of the rabbit (see Table 2) but so far we are not able to account for this fact.

The results of the experiments thus show that from the 12th day after inoculation a fall takes place in the immunological reactivity of the animal affected by a Brown-Pearce tumor; this is manifested by a fall in the titer of normal antibodies. At the same time, as was pointed out above, this is the period at which the tumor begins to give rise to metastases. It can be concluded from these findings that the onset of the process of metastasization of the tumor is connected with the fall in the immunological reactivity of the animal affected by the tumor.

The question whether in cancer patients there is immunity to the developing tumor is so far unanswered; different investigators have obtained contradictory results on this subject.

It has been established in the present investigation that production of anticarcinoma antibodies to a developing and metastasizing Brown-Pearce carcinoma does not take place in the rabbit. The results which we have obtained show also that the serum of these rabbits evidently contains normal antibodies, whose titer is not raised in the process of development of the tumor, but, on the contrary, begins to fall on the 10th-12th day. At the moment of death of the animals the normal antibodies disappear completely. The fall in the titer of normal antibodies coincides with the moment of appearance of metastases and the onset of violent growth of the tumor at the place of inoculation.

From the results obtained it can be postulated that the normal antibodies of a diseased rabbit play a definite part in the defense of the body against a developing tumor only in the first stages of its development, and for verification of this hypothesis special experiments are required.

SUMMARY

It was established in experiments conducted on 22 chinchilla male rabbits that normal antibodies may be revealed in the blood serum of healthy animals by the cold complement fixation test. In transplantation of Brown-Pearce's tumor the titer of these antibodies begins to decrease from the 10th-12th day, which coincides with the time of tumor metastasis. There is a complete disappearance of normal antibodies at the moment of the animal's death.

The author assumes that a definite role is played by the normal antibodies in combatting the developing tumor.

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THE EFFECT OF HIGH INTENSITY ULTRASOUND ON THE GROWTH AND METASTASIZATION OF INOCULATED BROWN-PEARCE TUMORS IN RABBITS

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The action of ultrasound on malignant tumors has attracted the attention of numerous research workers during the last decades. A number of workers abroad [9, 10, 13, 16-19], for instance, have tried out the action of low intensity ultrasound (2-4 w/ cm^2) on malignant tumors in animals. The results of these investigations are extremely contradictory. Attempts to use low intensity ultrasound in the treatment of malignant tumors in man, especially melanoma and sarcoma, have not been successful. Only individual workers [12, 17, 18] report the effective action of low intensity ultrasound on cancer of the skin in man. However, these findings were not confirmed by other authors [7, 8, 11, 14, 15].

In 1951 A. K. Burov put forward the hypothesis that these negative results are not due to the nature of the ultrasound but to the duration of its action at a low intensity, in consequence of which the thermal effect predominates. In the opinion of this author, it is possible by the use of high intensity ultrasound in short exposures to obtain positive results by their action on malignant tumors in animals and man [2, 3]. A. K. Burov constructed powerful ultrasonic apparatus capable of producing ultrasonic waves with an intensity of the order of 350 w/ cm^2 with flat quartz plates as a continuous system, and up to 500 w/ cm^2 as a pulsed system, i. e. ultrasound of ten times the limiting intensity obtained by other workers, and of 100 times that usually used by foreign workers in biological experiments.

The aim of our investigation was to study the effect of high intensity ultrasound on the growth and character of metastasization of malignant Brown-Pearce tumors in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits of the chinchilla breed, males, 2.5-3 kg in weight. The tumor

TABLE 1

The Effect of High Intensity Ultrasound on the Growth and Metastasization of a Brown-Pearce Tumor (Intramuscular Inoculation, First Group of Experiments)

Group of rabbits	No. of rabbits	Tumor present			Tumor absorbed
		widespread metastasisation	solitary metastases	metastases absent	
Experiment	41	9	2	3	27
Control	15	11	0	0	4

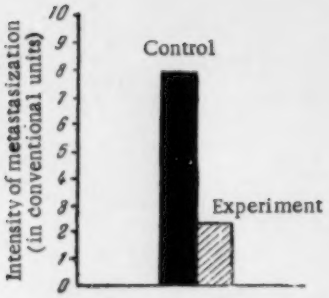

Fig. 1. Intensity of metastasization of a Brown-Pearce tumor in irradiated and control rabbits.

tissue was finely macerated and mixed with physiological saline in a proportion of 1:3, and this material was injected in a dose of 0.8-1 ml intramuscularly, intratesticularly, and into the testis brought down under the skin of the thigh and fixed in situ with a suture. This last method was undertaken to provide maximum transmission of ultrasonic energy to the tumor.

Animals were selected for the experiment on the 7th, 9th, 10th and 15th days after inoculation with the tumor, when the tumor nodule had reached a size of 4.5 × 3.5 × 2 cm. At these times, as a rule, at necropsy metastases could be seen with the naked eye in the internal organs. Survival experiments were carried out. In individual cases the observations lasted for 2-3 years. Rabbits killed at the end of the experiment, and also those dying in the course of the experiment, were subjected to thorough pathological anatomical examination. The antitumor activity of the ultrasound was determined by comparison of the experimental and control groups of animals, using the following criteria: the number of rabbits in which the tumors were becoming absorbed, the rate of growth of the tumor (according to its mean diameter), the duration of life of the animals, and the intensity of metastasization (in conventional units).

Altogether 204 rabbits took part in the experiment; 136 of them were irradiated under different conditions, and 68 acted as controls.

EXPERIMENTAL RESULTS

Depending on the method of inoculation and the conditions of action of the ultrasound, the experiments could be subdivided into 5 main groups.

In the first group of experiments (41 experimental and 15 control rabbits) we tested a single exposure to the action of ultrasound with a frequency of 718, 750, 1000 and 1500 kcps, an intensity of 22-35 w/ cm^2 and exposure time of 30-75 seconds (given fractionally) on a Brown-Pearce tumor inoculated intramuscularly (contact method of ultrasonic irradiation). Under the conditions of its action as enumerated above the effectiveness of the ultrasound was approximately the same in the individual experiments, and so we will consider the analysis of the results as summarized in Table 1.

The difference found between the experimental and control values is statistically significant, since T = = 3.92, i. e. is greater than 3.*

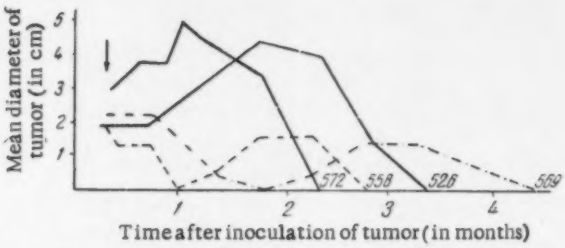

Fig. 2. Curves showing absorption of a Brown-Pearce tumor in irradiated and nonirradiated rabbits.
— nonirradiated rabbits (Nos. 572, 526); ----irradiated rabbits (Nos. 558, 568); the arrow indicates ultrasonic irradiation.

* T is the significance of the difference, calculated according to the formula quoted in A. A. Sapegin's "Variational Statistics" [6], where the difference is significant if T > 3.

TABLE 2

The Effect of High Intensity Ultrasound on the Growth and Metastasization of a Brown-Pearce Tumor (Inoculation into the Testicle, Second Group of Experiments)

Group of rabbits	System of irradiation			No. of rabbits	Results		
	frequency, kcps	power, w/cm²	exposure, sec		Tumor present: widespread metastasisation	Tumor present: restricted metastasisation	tumor absorbed
Experiment	1 500	35	4×15	5	1	3	1
	1 500	35	45	6	4	1	1
Control	Not irradiated			10	10	0	0

* In 4 rabbits, the nonirradiated metastases were absorbed.

TABLE 3

The Effect of High Intensity Ultrasound on the Growth and Metastasization of a Brown-Pearce Tumor (Intratesticular Inoculation, Third Group of Experiments)

Group of rabbits	System of irradiation			No. of rabbits	Results		
	frequency, kcps	power, w/cm²	exposure, sec		Tumor present: widespread metastasization	Tumor present: restricted metastasization	tumor absorbed
Experiment	1 500	27—37	17	13	4	3	6*
	1 500	27—37	12	12	11	0	1
Control	Not irradiated			16	15	1	0

* In 4 rabbits the nonirradiated metastases were absorbed.

As seen from Table 1, of the 41 experimental animals the tumor was completely absorbed in 27 (65.9%). In 5 animals dying from various causes the tumor disintegrated and was found in various stages of absorption. Metastases from these tumors were either absent or were found in insignificant numbers, in sharp contrast to the number of metastases in the control animals. Thus the number of metastases in 1 experimental rabbit was 2.56 conventional units and in 1 control rabbit – 8 conventional units (Fig. 1). In 73.3% of cases the control rabbits died of metastases.

Prolonged observation of the rabbits showed that the irradiated tumor was absorbed at different times after the procedure – from 7 days to 3½ months.

In the majority of experimental rabbits the tumor was absorbed between 20 and 40 days after ultrasonic irradiation. The process of absorption of the tumor in rabbits treated by ultrasound was, in the majority of cases, sharply distinguished from that of spontaneous absorption, which takes place in tumors inoculated by this means (Fig. 2).

As seen from Figure 2, at the end of the 1st-2nd month after ultrasonic irradiation disappearance of the tumor is observed, whereas in those control rabbits in which regression of the tumor takes place, this is the time when the tumor is at its largest size. Spontaneous absorption of the tumor ends 2-3 months after inoculation, and in place of the original absorbed tumor there arises afresh a thickening which grows for 1½-2½ months and finally is absorbed after 3-4 months.

As we have previously reported [5], in the majority of rabbits in which tumors have absorbed after ultrasonic irradiation, absence of metastases has been observed, and in some cases destruction and absorption of metastases formed in the lungs, kidneys, adrenals and the popliteal lymphatic glands has been noted.

TABLE 4

The Effect of High Intensity Ultrasound on the Growth and Metastasization of a Brown-Pearce Tumor (Intratesticular Inoculation, Fourth Group of Experiments)

Group of rabbits	System of irradiation			No. of rabbits	Results		
	frequency, kcps	power, w/cm²	exposure, sec		Tumor present: widespread metastasization	Tumor present: restricted metastasization	tumor absorbed
Experiment	1 500	80—150	3—1,3	21	10	1	10
Control	Not irradiated			17	16	1	0

A defect of the contact method of ultrasonic irradiation and the systems used was the gross damage to normal tissues (skin, muscles, bones) through which the ultrasound passed.

In the second group of experiments (11 experimental and 10 control rabbits) ultrasonic irradiation was carried out on rabbits with a tumor inoculated intratesticularly, in a water bath. It was found that this system (frequency – 1500 kcps, power – 35 w/cm², exposure – 60 seconds, given fractionally) which causes absorption of an intramuscularly inoculated tumor, slightly inhibits the course of the malignant lesion when inoculated into the testicle. Thus the average duration of life of the experimental rabbit was 81 days and of the control – 47 days; the average number of metastases in 1 experimental rabbit was 7 conventional units, and in 1 control rabbit – 14.5 conventional units. Absorption of the tumor was observed in only one experimental rabbit (Table 2).

Taking into consideration the more malignant course of a tumor when inoculated intratesticularly, we replaced the fractional method in this group of experiments by a continuous exposure (45 seconds). This led to severe necrosis of the irradiated testicle with the tumor and to its subsequent partial amputation. During this process metastasization was not halted and the majority of the animals died from metastases at the same times as the controls (see Table 2).

In the third group of experiments (13 experimental and 11 control animals) a reduction in the time of irradiation to 17 seconds at a frequency of 1500 kcps and a power of 27-37 w/cm² (transmission of energy through water) led to absorption of the tumor inoculated into the testis, both by the usual method and when transposed under the skin. Of the 13 animals the tumor was completely absorbed in 6 (46%) from 1-3-4 months after ultrasonic irradiation (Table 3). Tumor nodules which were palpable before irradiation, along the course of the spermatic cord, were absorbed 2 months after the procedure; metastases in the eyes, kidneys and lymphatic glands, which at first attained a considerable size, disintegrated and were absorbed within 3-7 months after ultrasolic irradiation of the primary tumor nodule. All the control rabbits died from multiple metastases from 1-1½ months after inoculation of the tumor.

However, even an exposure of 17 seconds, at an ultrasound intensity of 27 w/cm² was found to be too long, since it led to severe heating of the surrounding normal tissues (skin, bone) and to consequent trauma. A reduction in the time of irradiation to 12 seconds, with the intensity as before, while it reduced the damage to the normal tissues, it did not produce a positive effect (of 12 irradiated rabbits, 11 died from metastases – see Table 3).

The experimental results shown in Table 3 are statistically significant (T = 4.5).

In the fourth group of experiments (21 experimental and 17 control rabbits) the severe reduction in the length of exposure to 3-1.3 seconds, with a simultaneous increase in the intensity of the ultrasound to 80--150 w/cm² at a frequency of 1500 kcps (irradiation in a water bath) did not damage the normal tissues and led to absorption of the tumor in 40-60% of cases within 1½ -3-4 months after ultrasonic irradiation (Table 4).

Analysis of the results of the experiment as shown in Table 4 showed them to be statistically significant (T = 3.7, i. e. greater than 3).

In a number of the experimental rabbits disintegration and absorption of the nonirradiated metastases

formed in the eyes, kidneys, adrenals, lymphatic glands and bones were observed (these facts were confirmed by pathological anatomical examination [5]).

It was demonstrated by observation of the rabbits which had been cured, over a period of 2-3 years, that there was no recurrence of the tumors. Special experiments which we carried out showed that rabbits in which tumors had absorbed following ultrasonic irradiation were insusceptible to further inoculation of Brown-Pearce tumors [4].

In the fifth group of experiments on 48 rabbits (38 experimental and 10 control rabbits) we tested the action of other frequencies of high intensity ultrasonic irradiation (80 w/cm^2). The irradiation was carried out in a water bath. It was found that ultrasound of a frequency of 1000 kcps (exposure 2-7 seconds) did not give a positive effect, and of a frequency of 2000 kcps (with the same exposure) led to hemorrhages in the internal organs, the brain and the spinal cord, and caused death of the animals from 1-4 days after irradiation.

On the basis of the results obtained it was established that a single application of intensive ultrasound leads as a rule in 40-50% of cases, and in individual experiments in 60-70%, to absorption of a Brown-Pearce tumor inoculated either intramuscularly or intratesticularly into rabbits.

The fate of the tumor is dependent on the conditions and method of ultrasonic irradiation: a) ultrasonic irradiation at a frequency of 1500 kcps and an intensity of 27-37 w/cm^2 and with an exposure of 17 seconds leads to absorption of the Brown-Pearce tumor in a number of cases, but at the same time the adjacent normal tissues (skin, bone) suffer damage; b) brief exposure (3-1.3 seconds) to high intensity ultrasound (80-150 w/cm^2) of a frequency of 1500 kcps selectively acts upon the tumor tissue without harming the adjacent normal tissues.

Absorption of the irradiated tumor is accompanied by disintegration and absorption of the nonirradiated metastases in a number of cases.

SUMMARY

The author studied the effect of ultrasonic waves of high intensity on the growth and metastasis of Brown-Pearce's tumor. The rabbits were acted upon by sound waves in 7-15 days after inoculation of the tumor.

It was shown that single action by ultrasonic waves of high intensity (80-150 w/cm^2) at the frequency of 1500 kcps of short duration (3-1.3 seconds) results in the resolution of the Brown-Pearce tumor inoculated into the muscle or the testicle in 40-50% of the cases (in certain experiments in 60-70% of the cases) in the surrounding normal tissue is not disturbed under these conditions.

The resolution of the tumor acted upon by the ultrasonic waves is connected in a number of cases with the disintegration of metastases which were not acted upon by sound.

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THE EFFECT OF ASEPTIC PROLIFERATIVE INFLAMMATION ON THE LOCALIZATION AND GROWTH OF SPONTANEOUS TUMORS OF THE MAMMARY GLANDS IN MICE OF THE HIGH-CANCER C_3HA STRAIN

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There is insufficient reliable information at the present time to be able to assert that inflammation or an inflammatory reaction retards the growth of tumors. With a view to further study of the effect of inflammation on the growth of tumors we carried out experiments on mice of the C_3HA strain, which we obtained from R. E. Kavetskii's laboratory in Kiev. Tumors in mice of this strain are caused by a virus-like agent, which is transmitted along the maternal line through the milk of the lactating females; this agent has been called "milk factor". According to the observations of N. M. Turkevich [13], almost all female mice of this strain develop spontaneous cancer of the mammary gland at the age of 7-8 months.

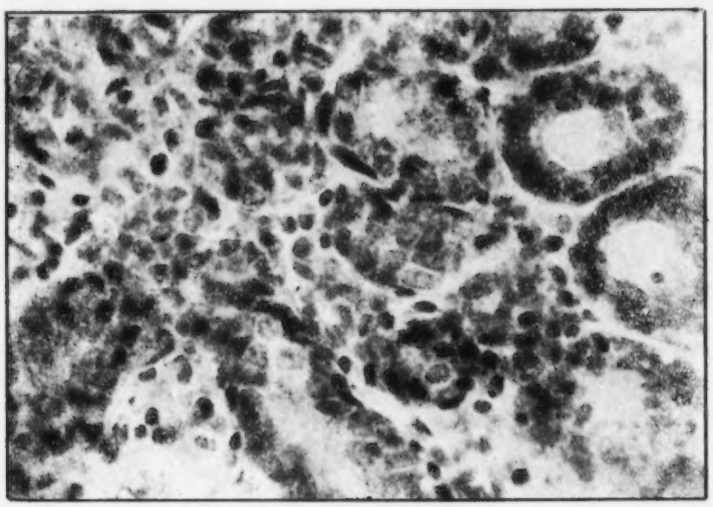

Fig. 1. Proliferation of the glandular epithelium of the mammary gland of a female mouse. Magnification 500.

In spite of adequate study of the etiology and the histological structure of spontaneous tumors in this strain of mice [4, 5, 13, 14, 15, 16], extremely little attention has been given to ascertaining the effect of inflammation on the growth and the course of spontaneous tumors of the mammary glands. The investigation of the influence of inflammation on the localization and growth of the spontaneous carcinoma of the mammary gland of virus

origin is of particular importance nowadays, when the virus etiology of tumors is receiving ever-increasing attention in both the Soviet and foreign literature [2, 3, 10, 11, 12, 16 and others].

EXPERIMENTAL METHOD

For the production of a proliferative inflammatory lesion, an injection of 0.3 ml of a 10% solution of an emulsion of infusorial earth in peach oil was given through a fine needle into the region of the left mammary gland of the fifth posterior pair in female mice at the age of 3 months. From 2-3 days later some swelling and erythema of the skin were observed at the site of injection.

One month after the injection of the 10% solution of emulsion of infusorial earth, at histological examination of the mammary gland a sluggish proliferative inflammatory lesion was observed around the acini and ducts of the mammary gland. Proliferation of young connective tissue cells (histiocytes, fibroblasts, polyblasts) and endothelioid cells, and accumulations of reticular cells and segmented polymorphonuclear leucocytes were seen. A well-marked proliferation of the glandular epithelium of the mammary gland was observed. The glandular epithelium was swollen and its apical part palely stained, and containing fine granular inclusions. In the lumen of the glands were masses of small granules, stained a pink color, and desquamated cells of the glandular epithelium. Histological diagnosis – a proliferative inflammatory lesion of the mammary gland (Fig. 1).

At the age of 5 months the same female mice were injected once again in the same region (corresponding to the fifth posterior pair of mammary glands, on the left side) with 0.3 ml of the 10% solution of the emulsion of infusorial earth in peach oil. The experiment was performed on 80 female mice. Eighty female mice of the same strain, which were not injected with the 10% solution of the infusorial earth emulsion into the mammary gland, acted as controls. It should be mentioned that the experimental and control mice were kept in identical conditions.

EXPERIMENTAL RESULTS

As seen from the figures in the Table, in the experimental group consisting of 80 mice spontaneous tumors of the mammary glands were seen in 61 mice, and in the control group adenocarcinoma of the mammary glands was found in 53 mice. In the remaining mice no spontaneous neoplasms were found after observation for two years. In the experimental group of mice multiple spontaneous neoplasms of the mammary glands were most commonly seen, in contrast to the analogous group of control mice. Multiple tumors were seen in 18 cases in the experimental group of mice, and only in 7 cases in the control group.

Still clearer was the difference between the experimental and control groups of mice in respect to the incidence of lung metastases from an adenocarcinoma of the mammary gland. Multiple metastases from carcinoma were observed in the lungs of mice of the experimental group in 17 cases, and in the control group of mice in only 8 cases. The sluggish inflammatory proliferative lesion also had a perceptible influence on the time of appearance of the spontaneous tumors of the mammary gland. The times of appearance of the first tumors in the mammary gland of the experimental and control groups are shown in Figure 2.

From the results given it follows that the greatest number of mice affected by tumors in the experimental group was at the age of 8-10 months. At the age of 8 months, for instance, spontaneous adenocarcinomas of the mammary gland developed in 12 mice, and at the age of 9 months – in 21 mice. In the following months of life of the mice the tumors developed more rarely. It must be pointed out that the first tumors in the experimental

The Influence of Proliferative Inflammation on the Growth of Spontaneous Tumors of the Mammary Glands

Group of mice	Total no. of mice	Number of mice			
		with spontaneous tumors	with multiple cancerous lessions	with metastases in the lungs	no tumor found
Experimental	80	61	18	17	19
Control	80	53	7	8	27

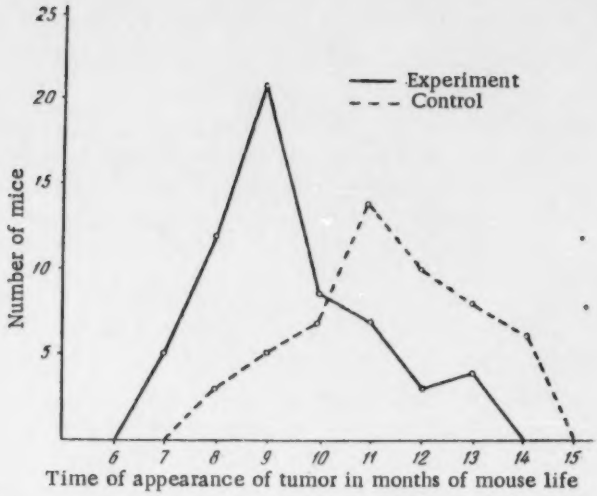

Fig. 2. Curve showing the appearance of spontaneous tumors of the mammary gland in mice of the high-cancer C_3HA strain, in relation to age.

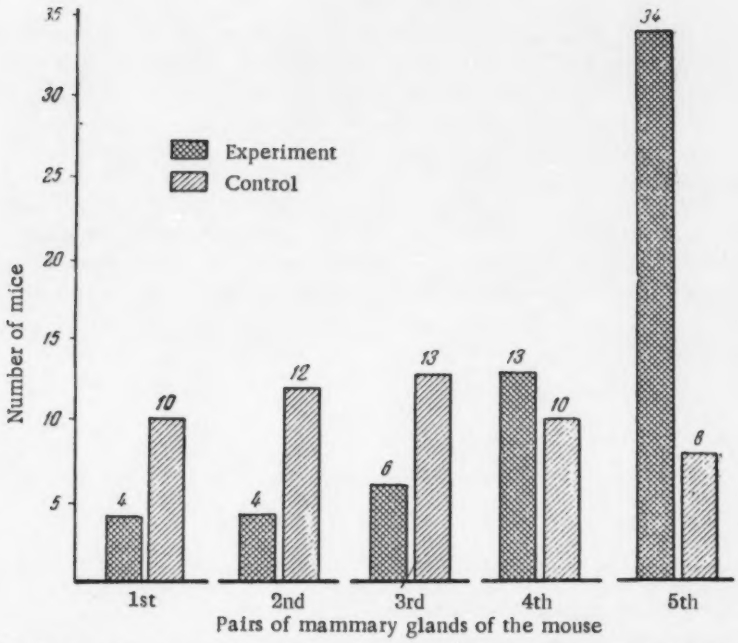

Fig. 3. Localization of spontaneous tumors of the mammary glands in mice.

Note: Where multiple carcinoma was present in 4 mice, only the location of the first tumor to appear is shown on the Figure.

group of mice were apparent at the age of 7 months, but in the control group of mice the first tumors were apparent only at the age of 8 months.

In the control group the greatest number of mice was affected with tumors at the age of 11-12 months.

At the age of 11 months tumors appeared in 14 mice, and at the age of 12 months – in 10 animals. From the results obtained it follows that a sluggish inflammatory process causes a number of changes in the body, bringing about earlier development of spontaneous tumors than occurs in the group of control mice.

The sluggish inflammatory lesion also had a perceptible effect on the localization of the spontaneous tumors of the mammary glands (Fig. 3).

The findings shown in Figure 3 show that spontaneous tumors developed most commonly in the region of the fifth pair of mammary glands, where the sluggish proliferative inflammatory lesion had been produced. In the experimental group of animals tumors appeared in the posterior pair of mammary glands (4th and 5th pairs) in 47 of 61 mice; in the anterior pairs of mammary glands spontaneous adenocarcinomas were found in only 14 cases. In 53 control mice with spontaneous tumors, adenocarcinomas were discovered in the posterior pairs of mammary glands (4th and 5th pairs) of 18 mice, and in the anterior pairs (1st, 2nd and 3rd pairs) of 35 mice. It must be pointed out that of the 34 mice in which spontaneous neoplasms arose in the region of the 5th pair of mammary glands, in 28 cases the tumors were found in the region of the left gland, where the proliferative inflammatory lesion had been produced.

The experiments carried out on 160 mice of the C_3HA strain give us the right to state that a proliferative inflammatory lesion has a perceptible influence on the localization and time of appearance of spontaneous tumors of the mammary glands. The neoplasms in the mice with an inflammatory focus appeared at an earlier age and were more often multiple in form than was the case in the control group of animals. The experimental results obtained showed that for the pathogenicity of the virus causing spontaneous tumors of the mammary glands in mice of the C_3HA strain to become apparent, suitable conditions are necessary. One of the more important of the conditions for the development of tumor viruses is probably the formation in the body of foci of cellular proliferation, which in our experiments were caused by sluggish proliferative inflammatory lesions.

SUMMARY

Experiments were performed on 160 female mice of the highly cancerous strain C_3HA with the so-called "milk factor". These experiments were aimed at studying the effect of proliferative inflammation on the growth of cancer. This proliferative inflammatory process was induced by the injection of 0.3 ml of 10% emulsion of infusorial earth in peach oil. At the age of 5 months the same mice received a repeated injection of 0.3 ml of 10% emulsion of infusorial earth in peach oil into the same site. Experiments demonstrated that the inflammatory process considerably affected the localization and the time of appearance of the tumors of the mammary glands. The neoplasms most frequently occur in the mammary gland affected by the proliferative inflammatory process. These neoplasms appeared in younger mice in comparison with the analogous group of control animals. The author assumes that the foci of the cellular proliferation caused by the inflammatory process create favorable conditions for manifestation of pathogenicity of the virus causing spontaneous cancer of the mammary gland.

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EXPERIMENTAL BIOLOGY

FACTORS OF OSTEOGENESIS AND THE SECRETORY FUNCTION OF TRANSITIONAL EPITHELIUM

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The question of the character of the histogenetic influences of epithelium in the development of osteogenic tissue is still unsolved. Meanwhile the appearance of bone tissue in phylogenesis, in the form of scales and integumentary ossification, and the formation of rudiments of corresponding structures in the present-day forms are undoubtedly connected with the formative action of the epithelium [3, 9].

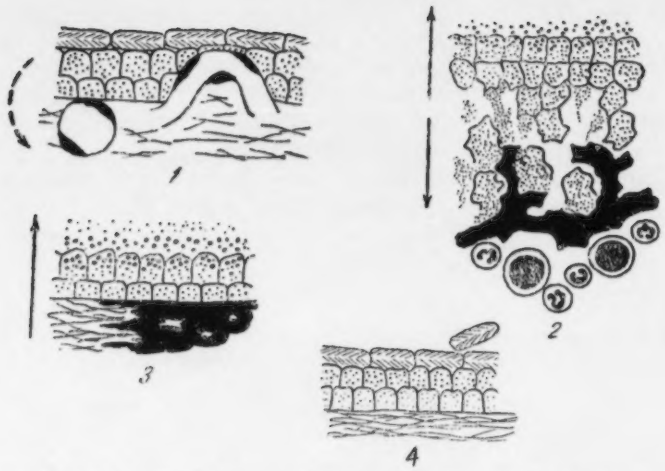
In this connection attention is drawn to the fact, known for some time, of the ectopic formation of bone near the transitional epithelium of the urinary organs – in the wall of the renal pelvis after ligation of the renal vessels [2, 12, 13] and in transplantates of the mucosa of the urinary bladder [11]. No satisfactory explanation of these facts exists.

In the present paper the results are given of an experimental, histogenetic, and histochemical analysis of these phenomena, which allow a possible explanation to be postulated.

EXPERIMENTAL METHODS AND RESULTS

The first series of experiments consisted of homografting of finely cut fragments of the mucous membrane of the urinary bladder of guinea pigs beneath the fascia of the rectus abdominis muscle. At times from 3 to 35 days the transplantates were fixed and, in addition to the routine histological study, examined histochemically for the detection of ribonucleoproteins, calcium salts, and polysaccharides [4-6].

Analysis of the histogenetic processes in the grafts enables one to determine the morphological expression of the osteogenetic action of transitional epithelium on connective tissue. It was found that large foci of osteogenic tissue, very reminiscent of typical rudiments of lamellar bones, are induced in the grafts. This induction is actually taking place on the 10th day after transplantation, and the differentiation of the connective tissue of the recipient into bone is maintained for 25 days, i. e. until the time of absorption of the transplanted homograft as a result of the immunological incompatibility of the tissues of the donor and recipient. Bone tissue develops in direct contact with the proliferating transitional epithelium, usually forming casings of bone around the epithelial cysts developing in the grafts. A morphological study showed definite signs for distinguishing those areas of the epithelial layer under which foci of osteogenesis were forming, i. e. active areas in the sense of their osteogenetic influence on the underlying connective tissue. Epithelium in a state of disturbed differentiation is active, but adult epithelium is not. The most characteristic form of relationship between this epithelium and the underlying connective tissue was found to be atypical growth of the epithelium with detachment of its cells from the layer, to become buried in the connective tissue, and also a series of other phenomena leading to the liberation into the connective tissue of the contents of epithelial cells at a definite level of differentiation. In the absence of these conditions mentioned, i. e. during normal differentiation of the transitional epithelium in the grafts, a basal membrane is formed between these and the underlying connective tissue, and the transposition of cells in the epithelial layer in the course of differentiation is effected from



Scheme of osteogenesis under the influence of transitional epithelium. 1 – Normal complete differentiation of transitional epithelium in the wall of the urinary organs; secretory activity possibly directed into the capillaries in intimate contact with the epithelium; surface of the epithelium formed by integumentary cells; secretion does not take place through the surface; 2 – atypical relationship between transitional epithelium and the underlying connective tissue during grafting and ligation of the renal vessels; secretion into the underlying tissue with induction therein of osteogenic and myeloid tissue; incomplete differentiation of the epithelium with absence of integumentary cells; secretion into the cavity of the cyst or the pelvis (secretion of pelvin); 3 – incomplete differentiation of epithelium with absence of integumentary cells from the wall of the cysts in the transplantates or in the renal pelvis during ligation of the vessels; epithelium lies on an already formed bony foundation or on dense connective tissue; secretion into the cavity of the cyst or the pelvis (secretion of pelvin); 4 – normal differentiation of transitional epithelium in the grafts; formation of integumentary cells and desquamation of these cells into the cavity of a cyst; development of a basal membrane underneath the epithelium.

the basal layer to the integumentary layer, so that the contents of the cytoplasm of the epithelial cells do not fall into the underlying tissue. Under these circumstances bone tissue never forms around these cysts, just as in the normal morphogenesis of the organs of the urinary system.

Histochemical detection of the polysaccharides (Schiff reaction) showed that the changes in the differentiation of the epithelium leading to osteogenesis are associated with disturbances of the polarity of the epithelial layer and the entry of intermediate products of metabolism into the underlying connective tissue [6].

In atypical conditions of growth in the grafts, polysaccharides from cells corresponding to cells of the transitional layer of epithelium are secreted into the underlying tissue. It is in these places, where there is secretion of polysaccharides into the tissue beneath from the epithelium, that the development of osteogenic tissue is induced. Histochemical reactions (Schiff reaction and for RNA) show that in addition to polysaccharides, substances of a protein nature, entering into the composition of the cytoplasm of cells of the intermediate layer of epithelium, are also secreted from the epithelium. Hence, it follows that the osteogenetic properties of transitional epithelium are probably due not to the appearance of new substances in it during transplantation, absent under normal conditions, but to the transfer from epithelium to connective tissue of certain substances which are probably always present in the epithelium but which usually do not reach the underlying connective tissue.

The above-mentioned changes in the differentiation of transitional epithelium, from what has been said, may be interpreted as evidence of some form of secretory process directed from the epithelium towards the underlying connective tissue; an osteogenetic factor is evidently secreted in the course of this secretory process taking place under the conditions of the experiment. In addition to the secretion into the underlying connective tissue just described, obvious secretion is observed in the grafts also, into the cavity of cysts through the surface of the epithelial layer where it is lacking in integumentary cells. The secretion consists of droplets of polysaccharide (not staining with mucicarmine) and a flocculent protein substance; as a result a translucent yellowish fluid accumulates in these cysts. This is not a question of secretion by individual goblet cells or their complexes, forming the glands of varying complexity in the composition of the mucous membrane, but of a secretory function of the whole epithelial layer. Secretion of mucus may also occur in the grafts, but induction of osteogenesis never takes place around such cysts [6].

In natural conditions the study of the secretory activity of transitional epithelium is made difficult by the fact that the products of secretion, if this takes place in the direction of the interior of the urinary organ, must be dissolved and excreted in the urine. This difficulty may, however, be overcome experimentally.

In a second series of experiments the vessels of the left kidney were ligated in rabbits, followed by ligation of the ureter in its lower third or by division of the ureter and suture of its proximal and distal ends to fatty tissue. Ligation of the vessels causes cessation of urine formation in the kidney and degeneration of its medullary and cortical layers. Viability is preserved only of the transitional epithelium of the pelvis, which undergoes proliferative changes, since vascularization of this structure is effected by means of vessels ascending in the wall of the ureter, and this is not cut off by ligation of the renal vessels. In these conditions, as is known [2, 13, 12], the development of osseous and medullary tissue is induced in the hilum of the kidney. It might be expected that if the transitional epithelium secretes into the cavity of the pelvis, the secretion can be collected in the lumen of the ligated ureter emerging from the kidney whose vessels have been ligated. When the end of this ureter is fixed by a suture, the secretion must flow along it, down to the place where it is fixed; the place of fixation of the distal end of the divided ureter served as a control. The operation on the ureter was carried out from 4-15 days after ligation of the renal vessels. From 10-215 days after ligation of the vessels the animals were killed. The left kidney, the left ureter and the place of suture of its ends, after fixation, were examined histologically and histochemically for the presence of polysaccharides. Smears were made of the contents of the ligated ureter. A detailed histogenetic analysis of this material must await further reports.

As a result of ligation of the vessels considerable proliferative changes develop in the epithelium of the pelvis, and as early as the 16th day, in places where there is infiltration of the transitional epithelium into the underlying tissue, the development of bone and of myeloid tissue is induced in it. The morphological expression of the osteogenic activity of the epithelium is composed in general of the same phenomena which were described in osteogenesis in transplantates of the urinary bladder [7]. It is relevant to stress the close genetic relationship between osteogenic and hemopoietic tissue [1]. Their simultaneous induction cannot therefore give cause for surprise.

In the conditions created by ligation, secretory processes are clearly revealed in the transitional epithelium of the pelvis [7]. They are directed, in the first place, into the underlying connective tissue and they are brought about as a result of those atypical relationships which are established by the infiltration of the transitional epithelium into that tissue. In these places foci of osteogenesis appear on the 16th-18th day after ligation of the vessels. In the second place, in those situations where the epithelium is thickened on account of proliferation of the intermediate type of cells, but without the formation of typical integumentary cells, the secretion is directed through the surface of the epithelium into the cavity of the pelvis. Under these circumstances there is clearly seen secretion from the apical parts of the superficial cells of flocculent (in fixed preparations) masses of polysaccharide and protein, and also droplets of glycogen. Often from the same areas of the epithelial layer it is possible to observe both secretion into the cavity of the pelvis through the surface of the layer, and secretion of the same substances of polysaccharide nature into foci of osteogenesis in the underlying connective tissue. In such areas, on the 20th to the 200th day after operation (and possibly even longer) there takes place accumulation and secretion from the apical parts of the cells of the whole layer of discrete, large drops of secretion, giving an intense Schiff reaction which is not removed by amylase; the secretion does not stain at all with mucicarmine. After liberation from the cells, the drops of secretion lose their proper shape, and in the cavity of the pelvis they form a flocculent protein mass which also gives a positive reaction

for polysaccharides. The name "pelvin" is suggested for this secretion.

Ligation of the ureter, when carried out after ligation of the renal vessels, has no essential effect on the changes brought about by ligature of the renal vessels alone (secretion, formation of bone and bone marrow). However, under these circumstances accumulation of translucent yellowish contents takes place in the ureter above the point of ligation. Morphologically the secretion of this substance by the transitional epithelium of the pelvis can be demonstrated perfectly clearly. On the slides it is quite similar to the secretion whose production by the pelvis was described above and also to the contents of the epithelial cysts in transplantates of transitional epithelium, where osteogenesis is induced [6, 7]. From 3-5 days after aspiration with a syringe, the contents accumulate once again.

Suture in fatty tissue of the proximal end of the divided ureter leading from the kidney whose vessels have been ligated, leads to the formation, on the 20th day, at the site of fixation, of multinuclear symplasts filled with small granules of polysaccharide, and around these – the appearance of foci of myeloid hemopoiesis. Control suture of the distal end of the ureter did not cause any such reactions. When the proximal end of the ureter has been sutured the contents of the pelvis flow down along it and accumulate in the ligated ureter. It must be emphasized that the formation of bone marrow at the proximal end of the divided ureter cannot be explained in any other way than by the histogenetic action of the contents of the pelvis that have flowed down to that point. In its morphology this process is similar to the induction of myeloid tissue in the region of the pelvis; under these circumstances at the place of suture of the distal end of the ureter, whose stump is in no way different from that of the proximal end except that no pelvic contents reach it, the development of myeloid tissue does not take place.

The findings described show that in experimental conditions an obvious secretory activity is shown by transitional epithelium. This secretion may be directed into the cavity lined by the transitional epithelium, or into the underlying connective tissue. In the latter case the development of bone and of bone marrow is induced underneath the epithelium.

With all its variety the morphology of the secretory processes demonstrates the similar character of the secretion into the cavity and into the underlying tissue, which is observed both in the case of formation of cysts in transplantates of transitional epithelium and after ligation of the renal vessels. In other words it can be considered that substances causing osteogenesis and hemopoieses are present in the secretion (pelvin) which may accumulate in the cavity of the pelvis. The fact that drainage of the pelvic contents through the ureter into connective tissue can cause myelopoieses confirms this conclusion.

As already emphasized, secretion by the transitional epithelium into the cavity through the surface of the layer is effected by means of imperfectly differentiated epithelium, in particular in the absence of typical integumentary cells. In normal urinary organs in contact with urine the differentiation of the transitional epithelium in complete,and conditions for secretion through the surface of the layer are evidently not created.

There arises, however, the question of physiological analogs of this secretion from the epithelium into the underlying tissue,which is observed in experimental conditions. It seems unlikely that histogenetically active substances, important for the body, should disappear (run to waste) in the process of normal development of the transitional epithelium. In this connection some preliminary observations may be made, which will require further confirmation, but which even now may be found useful in an analysis of the histogenetic factors of osteogenesis and hemopoiesis.

We may postulate the presence of a secretory (or incretory) process, directed from the transitional epithelium of the pelvis into the capillaries. It is possible that under these conditions the transitional epithelium secretes a certain factor (corresponding to the active principle of pelvin), which possesses a histogenetic action in respect to myeloid or osteogenic tissue. This form of incretory activity of the transitional epithelium, in A. N. Studitskii's opinion, may be connected with the hemopoietic function which is performed by the kidney in the lower vertebrates and in the earlier ontogenesis of the higher. In this connection it is interesting to observe that the mesonephros and the metanephros of birds evidently contain some substance which has a skeletogenic activity [10], and also to bear in mind the intimate contact with the capillaries that is so characteristic of transitional epithelium.

From the point of view of the hypothesis which we have propounded, a satisfactory explanation could be obtained of experiments on the induction of bone and of bone marrow by the action of transitional epithelium,

which are not so far understood. It is obvious that certain experimental conditions (transplantation, ligation of vessels) disturb the natural mechanisms of absorption of the factor – pelvin – secreted by the transitional epithelium into the blood stream, and it is deposited locally in the underlying connective tissue, where it induces the corresponding histogenetic processes.

SUMMARY

The author conducted an experimental, histogenetic and histochemical analysis of the transitional epithelium bone-forming activity in the transplantates and in conditions of ligature of the renal blood vessels. It was demonstrated that in such conditions substances of polysaccharide origin are secreted by the epithelium into the underlying tissue, as well as over the surface of the epithelium layer. It is suggested to name this secretion "pelvin". The introduction of pelvin in the connective tissue causes the appearance of myeloid hemopoiesis in it. A hypothesis is put forward of the presence of the incretory function in the transitional epithelium. The explanation of its bone-forming activity in experimental conditions is suggested.

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THE MITOTIC BEHAVIOR OF THE EPITHELIUM OF THE UTERUS AT DIFFERENT STAGES OF THE SEXUAL CYCLE IN MICE

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At the present time great attention is being given to the study of the laws governing the mitotic behavior of the body. However, so far too little attention has been paid to the study of the extent to which the mitotic behavior may reflect the functional state of a particular organ.

In this connection it appears essential to study the character of the changes in mitotic activity which are associated with certain rhythmic processes. One of these processes, as is known, is the sexual cycle, brought about by hormonal changes in the body. In this respect it would be of particular interest to study the mitotic behavior of the epithelium of the uterine mucous membrane, where physiological regeneration is completed in a short time and attains a high degree of morphological expression.

In the literature there are only isolated and scanty details on this subject. In some textbooks, in the description of the changes in the epithelium of the uterus in rodents during the sexual cycle it is observed that a large number of cellular divisions is present in proestrus, and that these are absent in diestrus and metestrus. I. D. Rikhter [1] draws attention to the sudden appearance of a large number of mitoses in the proestrus stage and stresses that the process of mitotic division of the nuclei pursues an energetic course in estrus also, but this gradually weakens and continues to diminish in metestrus.

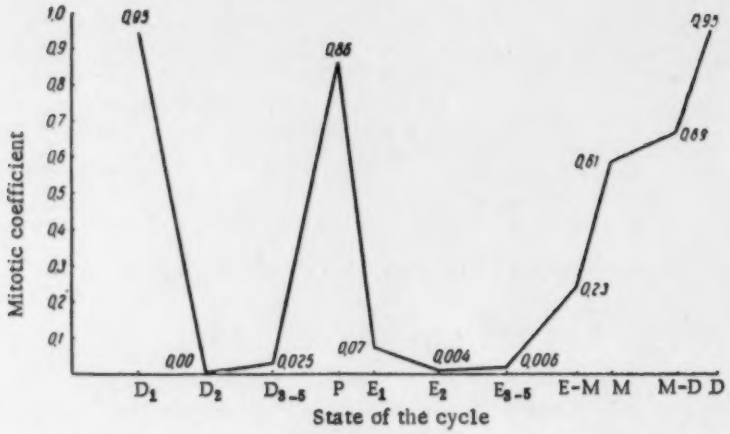
The quantitative characteristics of the mitotic activity of different organs in mice during the sexual cycle was the subject of an investigation by Bullough [5], who showed that the number of mitoses in the epithelium of the uterine mucosa is increased at the end of diestrus and in the stage of metestrus, and also after the injection of estrogens into the animal.

An increased number of dividing cells in the epithelium of the uterus in mice after injection of estrone was observed by I. A. Alov. In control experiments the peak period of cell divisions was found in the stage of diestrus and a smaller number was observed in estrus. Unfortunately, in this paper there is no distribution of the numbers of mitoses according to days during the longer stages (diestrus, estrus). Furthermore, none of the authors mentioned, with the exception of I. D. Rikhter, made a comparison of the mitotic activity of the epithelium of the uterus with its functional state. The lack of any quantitative counting of the mitoses in I. D. Rikhter's research prevents the formation of any definite conclusions on this question.

In the present work our aim was to demonstrate the character of the mitotic activity of the epithelium of the mucous membrane of the uterus throughout the sexual cycle and to compare the results obtained with the morphological changes in the endometrium.

EXPERIMENTAL METHOD

The investigation was carried out on 80 female white mice weighing 25 g. The stages of the sexual cycle were determined by taking vaginal smears twice daily for 10-11 days, after which the animals were



The mitotic coefficient in the epithelium of the mucous membrane of the uterus in mice at different stages of the sexual cycle.
D_1) 1st day of diestrus; D_2) 2nd day of diestrus; D_{3-5}) 3rd-5th days of diestrus; P) proestrus; E_1) 1st day of estrus; E_2) 2nd day of estrus; E_{3-5}) 3rd-5th days of estrus; E-M) estrus-metestrus; M) metestrus; M-D) metestrus-diestrus.

decapitated. The last smear was taken from each mouse immediately before decapitation. The uterus and ovaries were fixed in Zenker's fluid. In every case the upper third of the right cornu of the uterus was cut off with a razor for further treatment and embedded in paraffin wax. Transverse serial sections through the cornu of the uterus, 7-8 μ in thickness, were stained with hematoxylin and by Mallory's method.

In every fourth section (counting from the upper part of the cornu), the contours of the epithelium were traced under an Edinger drawing apparatus with a magnification of 178. For one case 10 such tracings were made. The mitoses were examined under the microscope (ocular 7 ×, objective 90 ×) and their distribution was indicated at the same time on the tracing of their particular section, mentioning the phase of division. The outlines of the tracing were next transferred on to standard paper, cut out with scissors and weighed on a torsion balance. From a knowledge of the weight of 1 cm^2 of paper the area of the epithelium could be determined. The intensity of cell division was judged by the changes in the value of the mitotic coefficient (the number of mitoses per unit area of epithelium).

EXPERIMENTAL RESULTS

The frequent taking of vaginal smears enabled it to be demonstrated that the overwhelming majority of experimental mice had a regular cycle, i. e. the appearance of all the stages of the sexual cycle in the animals was observed in sequence. However, the variations in the times of the various stages in different animals were very considerable. In addition in almost half the animals with a regular cycle, estrus lasted for as many or even for more days than diestrus.

The results obtained are shown on the Figure, where along the ordinate axis are represented the values of the mitotic coefficient (MK) and the abscissa represents the stages of the sexual cycle, with observation so far as possible of the time relationships between them. In consequence of variations in the times of the cycle in the mice, we grouped together the animals killed on the 3rd-5th days of diestrus and on the corresponding days of estrus. Besides this, on the abscissa are indicated signs for certain transitional stages of the cycle.

As the Figure shows, the mitotic activity in the epithelium of the mucous membrane of the uterus is not the same at different stages of the sexual cycle, and is expressed by a two-peaked curve with maximum cell division in proestrus and at the beginning of diestrus. The differences pointed out above in the duration of the separate stages of the cycle in the mice are not reflected in the character of the curve.

At the end of diestrus, in the later stages of which the mitotic coefficient was close to zero (MK = 0.025), a sharp increase takes place in the number of cell divisions in the epithelium of the mucosa (in proestrus MK = = 0.86), to be replaced by an equally rapid fall at the beginning of estrus. In the 1st day of estrus the mitotic coefficient is 0.07, and later on it falls still further, after which it remains practically unchanged throughout the whole period of estrus (MK = 0.004 and 0.006). During the transition to metestrus a gradual increase appears in the mitotic coefficient (MK = 0.23), which continues right up to the 1st day of diestrus, when the number of cell divisions is at its highest (MK = 0.95). However, on the 2nd day of diestrus the mitotic coefficient is already down to zero.

The changes observed in the mitotic activity correspond to those morphological changes which are undergone by the uterine mucosa during the sexual cycle.

The first rise in mitotic activity corresponds in time to the intensive proliferation of the uterine epithelium in the stage of proestrus. This stage is characterized by a rapid increase in the size of the uterus and widening of its cavity, the controus of which become broken up. At this stage the epithelial layer is clearly distinguished from the underlying tissue, the epithelial cells being high prismatic, with elongated and evenly stained nuclei, displaced to the basal part of the cell. The stroma is friable and contains numerous small glands with a well-marked lumen. The rapid course of the processes of proliferation of the uterine epithelium is replaced by an equally sharp fall in the tempo of cell division, which then remains low throughout the whole period of estrus. In this stage the uterus is still large, and its cavity is widened as before. The uterine glands attain a considerable degree of development. At the end of estrus the process of destruction of the epithelium begins: the nuclei become irregular in shape, they no longer stain evenly everywhere, and the phenomena of pyknosis and rhexis are often observed.

The second rise in mitotic activity in the transition to metestrus coincides in time with the process of regeneration of the epithelium and is accompanied by the gradual desquamation of its older areas. In metestrus the basal membrane, which sharply delineates the stroma from the epithelium, is visible only in isolated areas in the section. The nuclei of the epithelium become round, and move from their basal position to the center of the cell, sometimes even closer to its apical part.

At first the phenomena of pyknosis and rhexis of the nuclei are seen. The borders between the epithelial cells often cannot be made out, and in places the epithelium becomes stratified. At this stage the stroma is compact and consists mainly of round cells. The glands diminish in number and their lumen is narrow. The desquamation of areas of epithelium into the cavity of the uterus continues. The restorative processes in the uterine mucosa proceed more slowly than the proliferative processes in the epithelium in the stage of proestrus, and the rise in mitotic activity also takes place gradually.

After its reconstruction is complete, the epithelium of the uterus enters the stage of diestrus, i. e. one of relative rest. Its lumen becomes narrow and the epithelial layer is clearly divided by a basal membrane from the underlying tissue. The epithelial cells are cubical, and the nuclei are round or slightly elongated and stain evenly. Glands are present in extremely small numbers. Mitotic division of the epithelial cells is hardly observed at this stage.

In the course of the investigation it was found that the vaginal smears do not always accurately reflect the changes in the cycle taking place in the uterus. Thus the phase of proliferation begins in the uterus evidently sooner than in the vagina, and when the smear gives a picture of diestrus the endometrium is already showing the typical picture of proestrus. Other authors too [2] have drawn attention to this feature. At the end of estrus the changes in the vagina, on the other hand, precede the process of reconstruction of the epithelium of the mucosa; the smear is characteristic of diestrus whereas the uterus has not in fact entered the resting phase. It appears to us that this can explain the high mitotic activity of the epithelium in the first day of diestrus as determined by the smear.

In the study of the factors regulating cell division, great attention has been paid by investigators to the action of the sex hormones, in particular estrogens [1, 5]. The opinion is held that estrogens play an important role in the regulation of cell division in the reproductive organs and in organs directly connected with the reproductive system (mammary gland, pituitary).

It may be postulated that the rise in mitotic activity, which we demonstrated in the epithelium of the uterus at the stage of proestrus, is also due to an increase in the concentration of estrogens in the blood. However,

it is difficult to explain from this point of view the second wave of growth of cell division, beginning during the transition to metestrus.

An analysis which was made of the number of early and late phases of mitosis

$$\frac{EP + P + M}{A + T + LT}$$

where EP is the early prophase, P is the prophase, M the metaphase, A the anaphase, T the telophase and LT is the late telophase, showed predominance of early phases over late at all stages of the cycle. If, however, in the first day of diestrus and in proestrus, when the absolute number of cell divisions was at a maximum, the ratios between early and late phases of mitosis were 3 and 2.4 respectively, then during the transition to metestrus, and in metestrus they were 5 and 4.3, although the total number of dividing cells was several times smaller. Under these circumstances the relative number of metaphases hardly altered, varying in all stages between 30 and 40%. This increase in the number of early phases with an overall reduction in the number of mitoses suggests that besides a factor stimulating cell division there is apparent, at these stages, the action of an inhibitor, restraining the course of early phases of mitosis. Consequently, in this case the value of the mitotic coefficient does not evidently fully reflect the true mitotic activity of the particular meristem. This problem of the nature of the factors responsible for the peculiarities of cell division in the period of reconstruction of the epithelium of the uterus calls for special investigations. A stimulating effect of products of disintegration of the degenerating portions of the epithelial layer on this process is not excluded.

We may generalize from the results obtained to state that the changes in the mitotic activity of the epithelium of the mucous membrane of the uterus in the course of the sexual cycle bear a regular and rhythmic character and coincide in time with the morphological reorganizations of the mucous membrane which take place at the various stages of the sexual cycle.

It may be concluded from this that the mitotic behavior may reflect changes in the functional state of the organ.

SUMMARY

The mitotic activity of the epithelium of the uterine mucous membrane in mice differs at various stages of the sexual cycle and may be expressed by a double-peaked curve with the maximum cellular divisions in the proestrus and the initial stage of diestrus. The changes in the mitotic activity coincide in time with the morphological reconstructions of the mucous membrane at various stages of the sexual cycle. Consequently, the mitotic activity may reflect the functional condition of the organ.

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METHODS

A METHOD OF ONCOMETRY OF THE BRAIN

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In the experimental study of problems affecting the circulation of blood and CSF in the brain, when dealing with the question of edema and swelling of the brain the investigator makes use of the method of oncometry of the brain, which enables him to record the smallest variations in the volume of the organ and to trace them on the kymograph [1, 2]. In contrast to oncometry of other organs, in oncometry of the brain there is no need of a special bath in which to immerse the organ under examination; the function of this bath can be fulfilled successfully by the skull itself, with its perfectly hermetic walls [7, 8, 10]. Advantages of the method include its accuracy, simplicity and relative absence of trauma.

The simplest and undoubtedly the oldest method of recording the variations in the volume of the brain is by direct observation of the movements of the exposed brain. If, however, special devices are employed, the variations become more noticeable for observation. Sealing of a special oncometer hermetically in a drill hole in the skull, for instance, by making use of pneumatic transmission and a Marey's drum, enables the variations in the volume of the brain to be traced on a kymograph (the so-called pneumatic oncometer; [3-6, 8]). If the pneumatic transmission is replaced by hydrostatic, this dispenses with the necessity for hermetic sealing of the place of contact of the oncometer with the bone, and recording of the fluctuations of the brain is achieved by means of a glass funnel, the base of which is covered with a tightly fitting rubber membrane (water oncometer; [9]). The advantages and disadvantages of the various methods of oncometry of the brain are shown in the Table.

Variations in the volume of the brain undoubtedly represent one of the essential aspects of the dynamics of cerebral edema. By changes in the volume of the brain it is possible to judge reliably the successive stages in the development and course of cerebral edema, and its growth and diminution [10].

The method of oncometry of the brain which we have devised is as follows. In experiments of short duration on dogs under morphine-urethane anesthesia the right half of the vertex of the skull is exposed through a midline incision. A suture is passed through the substance of the temporalis muscle which is then divided at the base. In the parietal bone thus exposed a hole, 30 mm in diameter, is made by means of a hand-drill, and the dura mater is divided within the limits of this drill hole. Into this hole, after careful hemostasis, is inserted the cerebral oncometer, which is connected by a rubber tube to a water manometer for registration of the fluctuations of the brain on a kymograph.

The oncometer (Fig. 1) is a steel drum, the base of which is covered with a tightly fitting rubber membrane, while in the upper part is inserted a transparent acrylic plate through which the state of the brain can be freely observed. The diameter of the oncometer is 30 mm, to correspond to the diameter of the hole drilled in the skull; its height is 20 mm; the lateral surface is conical and is milled. The measurements of the cone are such that when the oncometer is inserted in the skull it does not penetrate deeper than the thickness of the bone, i. e. 2-3 mm. This allows firm fixation of the oncometer in the drill hole with support not on the brain but on bone. The upper part of the oncometer terminates in a projection which serves for attachment to the water manometer.

In order to obtain the most reliable curves in oncometry of the brain it is essential to ensure sensitivity

A Comparative Evaluation of the Methods of Oncometry of the Brain

Oncometry	Indications for use	Advantages	Disadvantages
Pneumatic	Registration of variations in the relative intracranial pressure	High sensitivity of recording of the variations in pressure	The necessity for strict hermetic sealing of the joint between the oncometer and the bone
Water	Registration of variations in the volume of the brain	No necessity to maintain strict hermetic sealing of the joint between the oncometer and the bone	Relatively lower sensitivity of recording of the variations

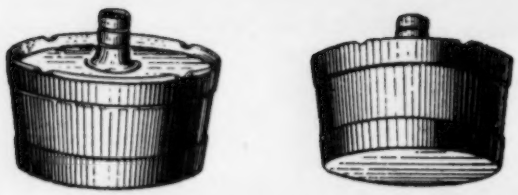

Fig. 1. The cerebral oncometer as constructed by the author. The base of the oncometer is covered with a tightly fitting rubber membrane.

of the recording system and accuracy of the registration itself.

The accuracy of registration of the variations in the volume of the brain is ensured by the following conditions:

1. Evenness and depth of anesthesia; changes in the level of anesthesia are undesirable since they lead to rapid changes in intracranial pressure.

2. Careful hemostasis; failure to observe this condition leads to accumulation of blood beneath the oncometer. In order to avoid bleeding the drill hole is made to the side of the midline (approximately three-quarters of a finger). Bleeding from the diploe is stopped with wax; vessels in the dura mater are caught with mosquito forceps and tied off.

3. Arrangement of the zero point of the manometer at the same level as the membrane of the oncometer (Fig. 2); the least disturbance of this relationship distorts the readings of the manometer.

4. Evenness of contact of the membrane of the oncometer with the surface of the brain in all parts of the membrane. This is achieved by observance of the following requirements: a) in selection of the animals puppies under 6 months old are not advised; at this age the brain does not entirely fill the cranial cavity in the regions close to the midline, and an even contact of the oncometer membrane with the brain can hardly be expected in these conditions; b) the oncometer membrane must be stretched evenly, without creases which would allow accumulation of fluid underneath the membrane; c) the oncometer must be fixed strictly in the plane of the drill hole in the skull; should it be inclined, erroneous manometer readings will be obtained.

Sensitivity of the recording system is ensured by observance of the following conditions:

1. Maximum shortening of the transmission system (the shorter the system the more sensitive it is) together with maximum lightness of the writing parts. The cork float of the manometer is carefully ground and coated with paraffin wax. The pivot of the float, as fine and short as possible, is cut from bamboo; the nib is cut from a piece of motion picture film.

2. Maximum cleanliness of the water manometer and of the liquid in it. The manometers used in the experiment are cleaned the night before with a chromic mixture and with distilled water.

3. Careful hermetic sealing of the whole system. This is filled with physiological saline (by a siphoning method). The hermetic sealing of the membrane is tested by inflating it under water. The material used for the

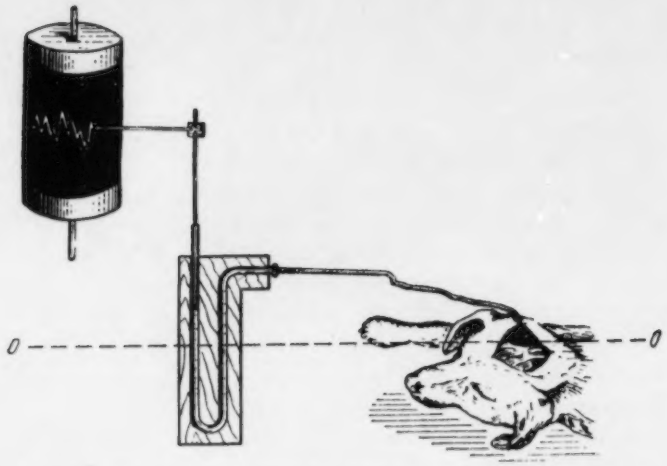

Fig. 2. Scheme of the experiment. The zero point of the water manometer is arranged to be at the same level as the membrane of the oncometer.

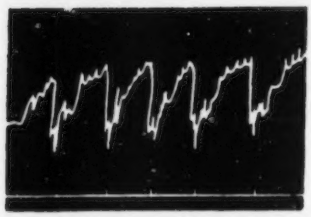

Fig. 3. Oncometric curve of the normal brain. Respiratory and pulse waves (more frequent) are seen.

membrane is thin rubber. Its tension is controlled as follows: a piece of rubber is placed over the base of the upturned oncometer, smoothed out and fixed without tension by means of two turns of thread to the rim of the oncometer. The rest of the rubber is cut off. While it is being filled with physiological saline the oncometer is placed with its base downwards on the edge of the table. Through the upper projection of the oncometer warm saline is injected by means of a syringe, so that it fills the interior of the oncometer, the projection and the rubber connecting tube. A clamp is placed on the latter, after which the oncometer is transferred to the skull and inserted in the drill hole, with gentle movement to the depth of two turns. In order to avoid damage to the membrane from the edge of the bone, the latter is carefully smoothed and the least roughness or traces of bone dust are removed.

After fixation of the oncometer the clamp is taken off the tube leading to the manometer and a recording of the fluctuations of the brain is made on the kymograph.

The normal curve of oncometry of the brain consists of a complex wave-like line (Fig. 3). Three types of periodic movement of the brain can be distinguished. In the most frequent rhythm appear the pulse waves, dependent on the activity of the heart. Respiratory waves have a slower rhythm and are formed as a result of the interaction of fluctuations in venous and arterial pressure. Finally, the slowest rhythm of all is shown by waves of the third order, or undulations, due to spontaneous processes in the vasomotor center.

A closer acquaintance with the kymograms demonstrates the enormous variety of types of oncometric curves, reflecting the complex dynamics of brain activity. A characteristic property of all the curves is the height of the initial level of the curve in relation to the zero line. As has been pointed out, the float of the manometer is arranged to be at the same level as the membrane of the oncometer, and in this position the zero line is marked on the paper on the drum of the kymograph. The subsequent removal of the clamp from the manometer is accompanied by a rise in the curve to a definite level which corresponds to the magnitude of the cerebral pressure (from 25 to 135 mm of water).

As the observations show, the level of the oncometric curve depends primarily on the degree to which the brain is filled with blood. Such manipulations as, for example, lifting the animal's trunk, or infusing into

its vein a large dose of isotonic solution, are accompanied by a rise in the oncometric curve. Conversely, manipulations leading to a reduction in the degree of filling with blood of the brain (for example, lowering the animal's trunk, hemorrhage, compression of the arteries of the neck) are accompanied by corresponding falls in the level of the oncometric curve.

Another essential factor determining the type and character of the oncometric curve is the act of breathing. In inspiration the oncometric curve of the brain is a line in a downward direction, and in expiration, a line directed upwards.

The amplitude of the oncometric curve is determined by the depth of the respiratory movements. The deeper the respiration the wider the range of respiratory movements of the brain, and the entire curve takes on the appearance of a bold, white band. On the other hand, shallow breathing is accompanied by very slight fluctuations of the brain, and in this case the curve has the appearance of a narrow band with small indentations. Holding the breath leads to disappearance of the respiratory excursions of the brain. Artificial respiration, carried out at this moment, is accompanied by restoration of the respiratory movements of the brain.

Pathological respiration of the Cheyne-Stokes, Kusmaul, etc. types is also reflected in the curve of oncometry of the brain. In Cheyne-Stokes breathing, for instance, periods of increasing amplitude of the respiratory movements alternate with periods of their diminution. After pauses of 3-4 seconds follow occasional superficial respiratory movements, gradually becoming much deeper and frequent. Having reached their maximum intensity, the respiratory movements again become shallow and infrequent. This type of breathing is associated, as we know, with lowered excitation of the respiratory center and it is observed in disorders of the cerebral circulation. In the period of infrequent respiratory movements the level of the oncometric curve characteristically rises; this is evidently associated with the development of stasis of the blood in the jugular and cerebral veins, thanks to the weakening of the aspirating action of the thorax during these periods.

An example of the combined effect of circulatory and respiratory factors is the oncometric curve of the premortal period. Most typically here there is a gradual and continuous fall in the level of the oncometric curve, parallel to the progressive fall in the arterial pressure, together with a weakening of the activity of the heart and respiration. A rarer type of oncometric curve in this period is a rising type with a maximum rise at the moment of death of the animal. An explanation of this phenomenon must evidently be sought in the appearance of congestive hyperemia of the brain. Thus the height and character of the oncometric curve of the brain are subject to fluctuation and this depends on the degree of filling of the brain with blood, the character of respiration and the general condition of the animal.

Results showing the possibility of using the method of oncometry of the brain in studying problems relating to edema and swelling of the brain, and also in elucidating the mechanisms of action of hypertonic solutions were given in our previous reports [1, 2].

SUMMARY

The author developed a method of oncometry of the brain in experiments on dogs. He used an original membrane oncometer. This method guarantees precise graphic registration of variations in the brain volume measured in mm of water. The method appeared to be useful in the study of the problems of cerebral circulations of blood and cerebrospinal fluid, edema and swelling of the brain, as well as in osmotherapy.

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A METHOD OF MEASUREMENT OF MUSCULAR TONE IN ANIMALS

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In experimental investigations on the study of changes in muscle tone it is desirable to use a method which would give accurate, quantitative readings, with as simple as possible a method of measurement. During investigations of the changes in muscle tone in experiments on frogs and cats, we used an indicator apparatus which combined simplicity of construction with accuracy. In experiments requiring measurement of the tension and the hardness of muscle it is advisable to discontinue the use of the spring which returns the pointer of commercial scales to its original position.

By means of this apparatus it is possible to carry out measurements of the changes in both length and hardness. In addition by means of two indicators it is possible to measure both these factors at the same time.

The scheme of the apparatus is shown in Fig. 1.

The object to be tested (a dissected muscle) is placed on a platform, the surface of which can move by means of screws in two mutually perpendicular directions in a horizontal plane.

The indicator in the apparatus which is in a vertical position (see Fig. 1, b) is used for measurement of the hardness of the muscle.

For this purpose a plunger 2 is screwed into the rod 1, its surface of contact with the muscle being covered with stearine. To the other end of the rod is fixed, by means of a screw, a small plate 3 for small weights. The rod is maintained in equilibrium by means of a counterpoise 4. In this position the plunger is just in contact with the surface of the muscle and exerts barely any pressure on it.

Before starting the experiment it must be checked that the rod with the attached plunger and small plate for weights are in equilibrium, and the rod moves freely inside the body of the apparatus.

In order to determine the hardness of the muscle, a weight of definite magnitude is placed on the plate 3, which depresses the rod and causes the plunger to compress the muscle. The pointer indicates the depth to which the plunger sinks into the muscle tissue. The deeper the plunger sinks as a result of the pressure exerted, or the lower the resistance of the muscle to the action of the weight compressing it, the less obvious is its hardness, which is one of the criteria of muscle tone.

In order to make the results of the experiments clearer it is essential to select a particular weight for each muscle. If the weight applied is too small it will fail to demonstrate differences in the hardness of the muscle under test in different conditions, since the small pressure will cause insignificant change in the shape of a harder or a softer muscle. The difference may be so small that it cannot be detected. If the weight applied is too large it may lead to considerable changes in the functional state of the muscle under examination. In practice, from experience, we usually used weights of 20-30 g.

Measurements of the tension of the muscle are made by means of an indicator fixed in the horizontal position (Fig. 1, a).

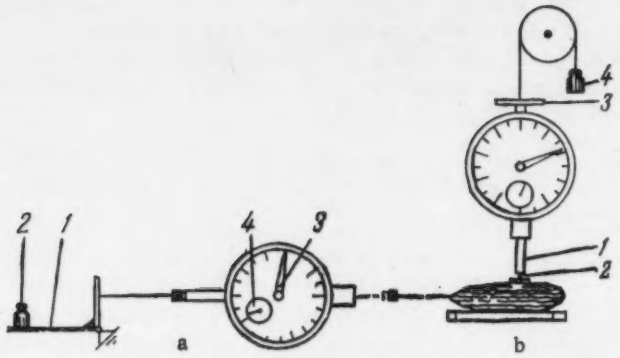

Fig. 1. Apparatus for measurement of the tension and hardness of muscles of animals.
a) Indicator in the horizontal position; 1) angular lever; 2) weight, acting as a counterpoise; 3 and 4) pointers indicating the degree of displacement of the rod; b) indicator in the vertical position.

Lengthening of the Thread (length 10 cm) by the Action of the Stretching Load

Load acting (in g)	Magnitude of stretching (in cm)	Load acting (in g)	Magnitude of stretching (in cm)	Load acting (in g)	Magnitude of stretching (in cm)
25	0	300	0	600	0
		400	0	700	0
50	0	450	0	800	0,05
100	0	470	0	900	0,06
200	0	500	0	1000	0,07

One end of the rod is connected to an angular lever 1, on which is placed a weight 2 acting as a counterpoise; it turns the lever 1, and with it the rod, back to its original position after shortening of the muscle (for example, at the moment of its contraction). The other end of the rod is attached to the muscle under examination by means of a thread.

The muscle is placed under tension by placing additional weights to the existing weight 2. The lever 1 then moves downwards and pulls the rod with it; this in turn stretches the muscle under test. The displacement of the rod, depending on the tension of the muscle, is indicated by the pointers 3 and 4.

After removal of the weight the muscle, returning to its original condition, shortens and pulls the rod with it, and its displacement is indicated by the pointers. Thus the length of the muscle is registered also at the moment of removal of the stretching load.

The lower the muscle tone, the more it will be stretched by the weight, and the greater the length of the muscle for a given load, and conversely the higher the muscle tone, the less it will be stretched and the shorter it will be for a given load.

The apparatus described is fixed to a firm universal stand.

By means of the stand and a platform it is possible to assemble the measuring apparatus sufficiently accurately in relation to the muscle to be tested without moving the animal from its place.

As already pointed out the muscle is connected to the apparatus by means of a thread. Control experiments showed that within the limits of the loads used in the determination of muscle tone (20-30 g), the thread is in practice inextensible (see Table).

When working with this suggested apparatus it is essential to bear in mind that for overcoming the force of friction arising during movement of the parts of the apparatus it is necessary to determine the force required to displace the mechanism from its state of rest. We determined the minimum value of the load which, when applied, would set the apparatus into action. For the measurer of the muscle hardness this amounted to 1-1.5 g, and for the measurer of the length of the muscle – 300-500 mg.

The construction of the apparatus enables simultaneous kymographic recording of the changes in hardness and extension of the muscle to be made by means of the ordinary Engelman levers at the same time as the measurements are indicated by the pointers.

As shown by experiments on animals (frogs and cats), it is possible by the use of this method to detect even insignificant changes in the tension and hardness of a muscle under the influence of various factors. In this way it was discovered that as a result of certain gross measures (for example, denervation, interference with the blood supply) the tension and hardness of muscles may be altered unequally, whereas under normal conditions the tension and hardness of muscles alter in a parallel manner.

SUMMARY

The author recommends a new method of measuring the muscle tone with the aid of a simple instrument – a modified indicator.

The method developed on frogs and cats gives precise quantitative readings, while the technique of the measurement is very simple. This method enables measurement of tensile strength and the hardness of the muscles simultaneously; kymography may be performed at the same time.

A METHOD OF CONTINUOUS RECORDING OF THE BLOOD FLOW IN THE BRAIN IN EXPERIMENTS OF SHORT AND LONG DURATION

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The method which we have used to record the temperature of the brain in an experiment of long duration in dogs [1] can be adapted for continuous recording of the blood flow. This is effected, just as in the method used by Gibbs [3] and Ludwigs [4], by supplementary heating of the thermoelectrode at its point of insertion, in order to raise its temperature above that of the brain and of the circulating blood, which allows the cooling effect of the blood flow on the temperature of the source of heat to be recorded and, consequently, the character of the local circulation to be judged.

However, the technical difficulties in the preparation of the thermoelectrode equipped with a special adaptor for supplementary external heating led us to make use of the properties of the thermistor itself for this purpose. If the supply to the bridge – one of the arms of which is the thermoelectrode – is increased, then as a result of a thermistor of very low power (about 20 μw) this will be heated. A change in the magnitude of the current flowing through the bridge may achieve heating of the thermistor to 1°C above the temperature of the circulating blood. Under these circumstances the current strength necessary to pass through the thermoelectrode

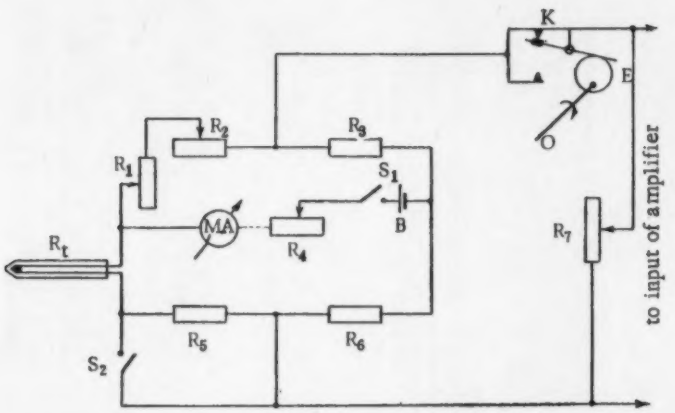


Fig. 2. Bridge circuit of the amplifier input.
R_1, R_2, R_3, R_6) Arms of the bridge; R_t) thermistor; R_4) rheostat (20 kiloohm) regulating the supply to the bridge; R_5) resistance standard; S_1) bridge supply switch; S_2) standard switch; R_7) rheostat (20 kiloohm) shunting the amplifier input; B) normal cell; K, E, O) mechanical interrupter; μA) microammeter.

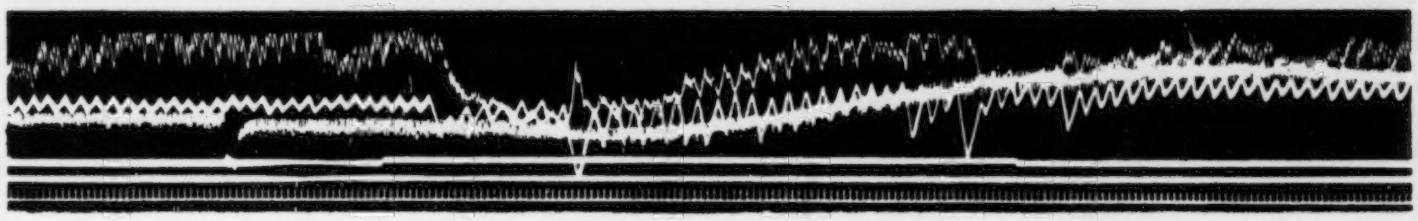
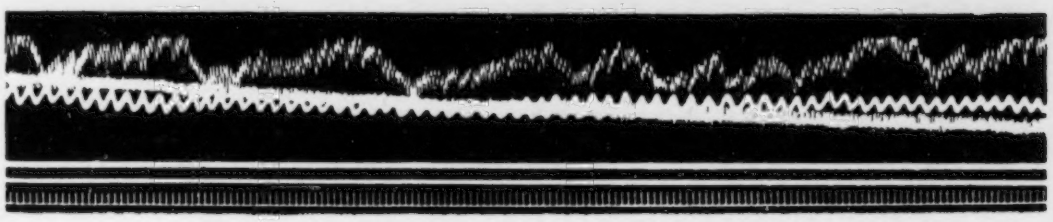


Fig. 1. Changes in the plethysmogram (tracing from the tail), in respiration and in the local cerebral circulation in the parietal region. Significance of the curves (from above downward): plethysmogram, respiration, cerebral circulation, stimulation marker, time marker.



is greater, the higher the density and the thermal conductivity of the surrounding medium (when using a thermistor with a resistance of approximately 1500 ohm, the nominal current strength passing through it must not exceed 110 μa; during registration of the blood flow the current strength is raised to 0.6 ma).

The simplicity of the adaptation allowing regulation of the supply to the bridge (Fig. 2) and the very considerable gain in sensitivity during registration of the blood flow by the thermistor included in such a circuit (6 times or over) are the advantages of this method. A similar method for registration of the blood flow in the uterus was used by I. I. Benediktov [1].

Figure 1 illustrates the effect of a change in the concentration of carbon dioxide in the expired air on the level of the blood flow in the brain in the region of the skin analysor.

SUMMARY

A change in the intensity of the direct electric current passing through the needle-formed thermoresistance (connected into the bridge circuit forming the input to the amplifier) raises the temperature of the thermistor to 1°C over that of the inflowing blood. This makes it possible to register the blood flow at various areas of the brain.

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A MODIFICATION OF THE METHOD OF DIFFERENTIAL MANOMETRY FOR REGISTRATION OF THE VOLUME VELOCITY OF THE BLOOD FLOW

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Several forms of apparatus for registration of the rate of the blood flow by the method of differential manometry are described in the literature [1-4]. They consist essentially of a cannula joining the proximal and distal ends of a divided vessel. The size of the lumen of the cannula is regulated by special cut-out plates with orifices of different diameters, or by screws. When the lumen is reduced, the linear velocity of the blood flow at the point of constriction increases, and the pressure falls in proportion to the reduction in the area of cross section of the lumen. The pressure difference before and immediately after the cannula, according to the Bernulli formula, is proportional to the difference of the squares of the velocities of flow at these points, and ultimately reflects the rate of the blood flow:

$$P_1 - P_2 = \frac{\rho v_2^2}{2} - \frac{\rho v_1^2}{2}, \tag{1}$$

where P_1–P_2 is the pressure difference; v_1 and v_2 are the linear velocities of the blood flow before and after the constriction; and ρ is the density of the fluid.

In another modification measurements are made of the difference between the lateral pressure and the pressure in a Pitot tube (a manometric tube turned to face the flow of blood). In this case the rate of flow in the Pitot tube v_1 is equal to zero, and the formula is simplified to:

$$P_1 - P_2 = \frac{\rho v^2}{2}. \tag{2}$$

The main drawbacks of these forms of apparatus are, in the first place, that where solid matter projects into the cannula, especially at points of constriction, sedimentation of blood cells takes place, with coagulation of the blood, altering the sensitivity and distorting the readings of the apparatus; in the second place, when the velocity of the blood flow is less than 50 ml/min the sensitivity of the apparatus is low. As shown by experimentally verified calculations, when registering the velocity of flow by the method of differential manometry of such a relatively viscous fluid as blood, then Bernulli's law is less applicable than Poiseuille's law:

$$P_1 - P_2 = V \frac{8\pi\eta l}{q^2}, \tag{3}$$

where η is the viscosity of the blood, l the length of the portion of vessel or tube; V the volume rate of flow in the portion of vessel. In consequence of this we used the following modification of the method.

A rubber tube 2 and 3 (Fig. 1), 2-3 mm in diameter is inserted into an artery of the organ to be tested.

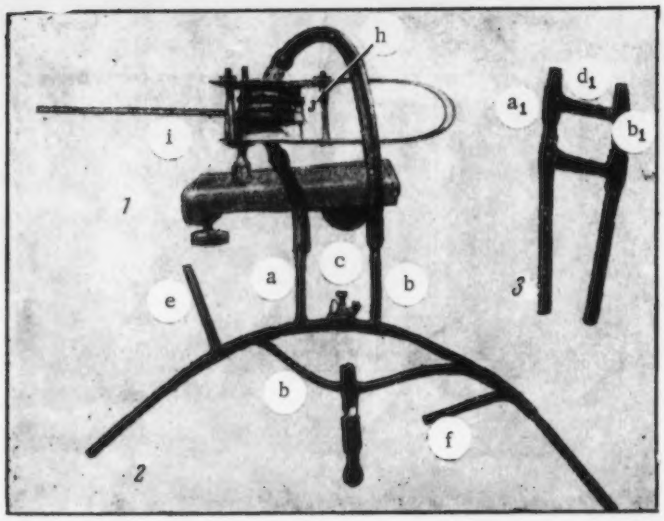

Fig. 1. Apparatus for registration of the magnitude of the blood flow. 1) Differential manometer; 2 and 3) rubber cannulas.

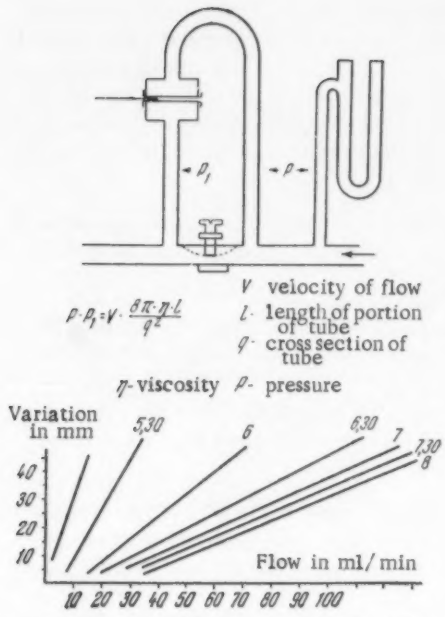

Fig. 2. Scheme of the apparatus and calibration graphs for different degrees of sensitivity of the apparatus.

The tube has two branches situated at some distance from each other (see Fig. 1, a and b or a_1 and b_1) and connected to the chamber of a differential manometer 1 (Fig. 1). According to Poiseuille's formula, the fall in pressure in the tube between the branches is proportional to the volume rate of flow of the blood. Since η, l, q, and consequently the value $8\pi\eta l / q^2$, expressing the resistance of the portion of the tube, are constant throughout the experiment, and the formula acquires the form:

$$P_1 - P_2 = Vk, \qquad (4)$$

i. e. the readings of the differential manometer are directly proportional to the volume rate of blood flow; $8\pi\eta l / q^2$ (the resistance of the portion of tube) is the coefficient of proportionality – k.

A screw clamp c (see Fig. 1) is applied to the tube between the branches, enabling the cross section of the tube to be varied. In this way k, i. e. the sensitivity of the apparatus, is changed in inverse proportion to the square of the section, q^2. In each experiment the clamp is placed in a definite position to allow adequate sensitivity of the apparatus for a given blood flow.

The apparatus is calibrated as a first step: graphs are prepared for different degrees of sensitivity showing the slope of the curve (in millimeters) in relation to the magnitude of the volume blood flow (in milliliters per minute). The graph is a straight line, the gradient of which increases as does the sensitivity of the apparatus (Fig. 2).

Calculation of the value of the slope starts from the zero level, i. e. from the level of the readings at equal pressures in both chambers of the manometer. For this reason, in the course of the experiment, it is

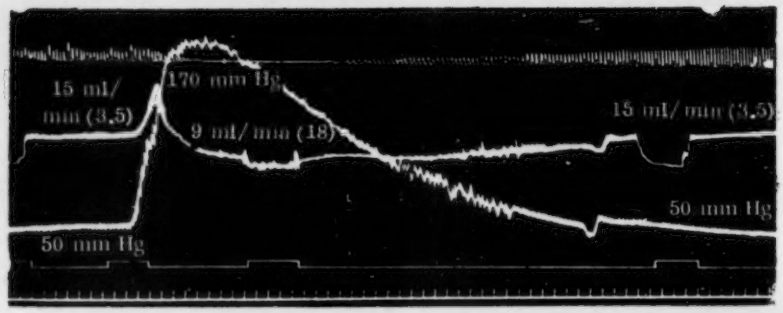

Fig. 3. Registration of the blood flow in the hind limbs of a cat during the intravenous injection of adrenalin (75 γ / kg). Significance of the curves (from above downward): respiration, blood flow, blood pressure, marker for injection of the drug and verification of the zero level, time marker (5 seconds). The sharp falls in the line registering the blood flow correspond to observations of the zero level. The figures in brackets show the resistance of the vascular bed of the limb.

essential to equalize the pressures in the chambers periodically (every 5-10 minutes) and to observe the zero level. This is done by including in the circuit a parallel tube d (see Fig. 1), or better still – by joining the two branch tubes a_1 and b_1 (see Fig. 1) with a cross tube d_1 (see Fig. 1), on which a clamp is applied. When this clamp is transferred to the branch a_1 or b_1 immediately below the cross tube d_1, the pressure in the chambers is equalized without any alteration in the conditions of the blood flow.

The differential manometer which we used 1 (see Fig. 1) was constructed from two small Marey's capsules (manometer chambers h; see Fig. 1), firmly fixed to each other by means of rubber membranes. Between the membranes is attached one arm of a lever with two arms, i (see Fig. 1). The axis of rotation of the lever is placed alongside the point of contact of the capsules, at the margins of which small, narrow cuts are made to allow movement of the lever. The second arm of the lever traces on the drum of the kymograph, either directly or through a system for magnifying the variations. During the experiment the entire system is filled with physiological saline.

Other and more sensitive systems of manometers (optical, electromanometers, and so on) may be used. Under these circumstances the coefficient k, i. e. the resistance, included in the blood flow can be reduced to a minimum.

Among the advantages of the proposed modification are the simplicity of the apparatus and the absence of hard, angular projections from the path of the blood, thanks to which there is no destruction of blood cells and no coagulation of the blood. In the course of an experiment lasting 4-5 hours the sensitivity of the apparatus stays constant. Another advantage of the apparatus is the adequate fall in pressure; with volume rates of blood flow of 10-50 ml/ min, it is several times higher than in the apparatus working on Bernulli's formula, with the same minimum diameter of cannula (approximately 2 mm).

As the experiments showed, the apparatus allows registration of the volume velocity of the blood flow – 10 ml/min and over. If the degree of sensitivity is arranged close to its maximum value (for example, 5, 30 on the graph; see Fig. 2), the deviation of the recording lever corresponding to a change in the volume velocity of 1 ml/ min is 1.5 mm. Such sensitivity readily allows registration of the changes in the blood flow in the limbs, kidneys, head and abdominal viscera of average laboratory animals (cats), not to mention the larger ones (dogs; Fig. 3).

A slight modification also allows Bernulli's law to be utilized (see Fig. 1, 3). The first branch a_1 is in practice a Pitot tube. In this way a useful combination of both methods of differential manometry can be brought about.

Finally, an advantage of the method described is the direct proportion between the manometer readings and the volume velocity of the blood flow.

In practice it is convenient to have two further branches from the tube: one for registration of the blood pressure (see Fig. 1, e) and the other (see Fig. 1, f), situated beyond the branches to the differential manometer, for injection of drugs directly into the arterial system of the organ under examination. The method is also applicable for registration of the flow of nutrient fluid in organ perfusion experiments.

SUMMARY

A modification of the method of differential pressure measurement for registering the volume velocity of the blood flow is described. A rubber tube is connected to the blood vessel. The difference in the lateral pressure exerted in its two consecutive points is, in accordance with the Poiseuille formula, proportional to the volume velocity of the flow. This difference is registered by a differential pressure gauge. The rubber tube has a screw clamp between the side branches connected to the pressure gauge, providing means of controlling the resistance of the corresponding section of the tube, and thereby the sensitivity of the whole instrument.

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